WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: CyFlow Counter System with CD4 easy count kit and CD4% easy count kit
WHO reference number: PQDx 0350-081-00

CyFlow Counter System with CD4 easy count kit and CD4% easy count kit with product codes **CY-S-3023, 05-8401, 05-8405, 05-8410, and 05-8411**, manufactured by **Sysmex Partec GmbH**, **CE-marked regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 8 August 2018.

Summary of WHO prequalification assessment for CyFlow Counter System with CD4 easy count kit and CD4% easy count kit

	Date	Outcome
Prequalification listing	8 August 2018	listed
Dossier assessment	24 May 2018	MR
Site inspection(s) of the quality management system	18 October 2021	MR
Product performance evaluation	October 2017 to February 2018	MR

MR: Meets Requirements

Report amendments and product changes

This public report has since been amended. Amendments may have arisen because of changes to the prequalified product for which WHO has been notified and has undertaken a review. Amendments to the report are summarised in the following table, and details of each amendment are provided below.

Version	Summary of amendment	Date of report amendment
2.0	Change of labelling in the safety data sheet, label and IFU for 04-4012 Hypochlorite Solution due to added H + P statement and signal word in the safety data sheet of the manufacturer of the raw material; as a consequence of adjustments to regulation on handling substances hazardous to waters (part of CLP Regulation (EC) 1272/2008)	16 October 2019
3.0	Change of storage temperature of the Sheath Fluid (Ref. No. 04-4007) from 2-25 °C to 18-30 °C. Sheath Fluid is an accessory	19 December 2019

	solution for flow cytometers operated in laboratories at room temperature and does not need to be kept refrigerated. In-use and shelf life stability were tested within the temperature range of 18-30 °C. Consequently, a change of labelling material was required. Change in type and material of buffer bottle caps in CD4 easy count kit and CD4% easy count kit due to leakage and insufficient practicability (Corrective action CA-18005). The change involved three steps: 1. Change in Product Immediate container - new closure cap for buffer components 2. Change of manual capping procedure for Machine-controlled capping using an automated production line. 3. Automated production line performs filling and capping.	
4.0	Fulfilment and closure of commitment for prequalification to further invest in a continued root cause analysis of the large negative bias observed between CD4 levels measured on the Sysmex instruments and using Sysmex assays as compared to measurements made using other CD4 measuring assays (e.g. FacsCalibur and MPL/CellMek with PLG).	30 April 2020
5.0	1. Substitution of Triton X-100 as part of the Count Check Beads green reagent produced by Sysmex Partec GmbH based on based on the REACH regulation that prohibits the use of TritonX-100 from 4 January 2021. 2. Updating of the product's labelling address and intended use according to IVDR. Sysmex Partec GmbH legal company address changed from Am Flugplatz 13, 02828 Görlitz, Germany to Arndtstrasse 11 a-b, 02826 Görlitz, Germany. 3. Amendment of the intended purpose according to IVDR of CD4 easy count kit and CD4% easy count kit.	21 June 2021
6.0	Change of product codes of IVDR Class A products (CyFlow Counter, Hypochlorite Solution, Count Check Beads green, Decontamination Solution, Cleaning Solution, Sheath Fluid).	12 August 2022
7.0	Relocation of production of CD4 easy count kit (05-8401) and CD4% easy count kit (05-8405) from the original site (EXBIO II & III) to the new manufacturing and logistics facility (EXBIO IV).	6 August 2024
8.0	IVDR Transition for CD4 and CD4% easy count kit (from IVDD to IVDR), including product code changes; CD4 easy count Kit (IVDD Product Code 05-8401 to IVDR product code 05-8410) and CD4% easy count kit (IVDD product code 05-8405 to IVDR product code 05-8411). Increased the shelf life from 14 to 16 months.	16 October 2024

9.0	Introduction of a filtration step (0.2 µm filter with PES membrane) during filling of the Hypochlorite Solution manufacturing, since the risk was detected that particles can get into the solution through the filling tap.	4 June 2025

Intended use

According to Sysmex Partec GmbH, "*the **CD4 easy count kit** is a two-component, quantitative IVD test for subpopulation labelling of lymphocytes in adult venous EDTA whole blood, and subsequent enumeration with a suitable Sysmex Partec IVD flow cytometer. The CD4 T cell concentration is useful to assess the immune and clinical status of patients. It is an indicator for the initiation or follow-up of treatment for people living with HIV, in conjunction with other laboratory and clinical findings. The test is intended to be performed by trained healthcare professionals and can be used for both manual sample preparation and automated use with a suitable Sysmex Partec sample preparation system."*
The ***CD4% easy count kit*** *is a four-component, quantitative IVD test for labelling of leukocytes and a subpopulation of lymphocytes in adult venous EDTA whole blood, which can then be enumerated with a suitable Sysmex Partec IVD flow cytometer. The CD4 T cell concentration and CD4% of lymphocytes in blood samples are useful to assess the immune and clinical status of patients. They are indicators for the initiation or follow-up of treatment for people living with HIV, in conjunction with other laboratory and clinical findings. The test is intended to be performed by trained healthcare professionals and can be used for both manual sample preparation and automated use with a suitable Sysmex Partec sample preparation system.*

*The **CyFlow Counter, accessory solutions, and assays** are intended as aid to diagnosis of immune and clinical status of patients and for monitoring, initiation or follow-up of treatment for people living with HIV. The CyFlow Counter is a manual cell analysis system designed for the determination of the absolute number of CD4+ T lymphocytes and the percentage of CD4+ cells in lymphocytes in human EDTA venous whole blood samples. The use of the CyFlow Counter is limited to healthcare professionals, trained by staff of Sysmex Partec GmbH or authorised distributors.*"

Assay description

According to the claim of Sysmex Partec GmbH, for "*the CD4% easy count kit, an aliquot of an EDTA whole-blood sample is mixed with two antibodies, each conjugated to a different fluorochrome for labelling dedicated cell populations. After a fixed incubation time, the two buffer solutions are added, and the sample is ready for analysis on the CyFlow Counter flow cytometer. The light source excites the fluorescent dye linked with the stained cell and the emitted light is detected while a certain volume of blood sample is running through the instrument. The concentration of the dedicated cell populations is calculated by the integrated software. For further information, please refer to the IFU of CyFlow Counter (CY-S-3023IFUEN).*

For the CD4 easy count kit, an EDTA whole-blood sample is mixed with the antibody conjugated to the fluorochrome in a 1:1 ratio. After a fixed incubation time, the buffer is added, and the sample is ready for analysis on the CyFlow Counter flow cytometer. The light source excites the fluorescent dye linked with the stained cells and the emitted light is detected while a certain volume of blood sample is running through the instrument. The integrated software calculates the concentration of the dedicated cell populations. For further information, please refer to the IFU of CyFlow Counter (CY-S-3022IFUEN)".

Test kit contents

Component	Product code	Test/Kit
Instrument		
CyFlow Counter (Flow cytometer)	CY-S-3023	N/A
Software CyView 2.11	N/A	N/A
CD4 easy count kit		
CD4 easy count kit to count absolute CD4+ T-lymphocytes	05-8401 05-8410	100 tests
Vial containing CD4 mAb PE	05-8401-01 05-8410-01	N/A
Bottle containing no lyse buffer	05-8401-02 05-8410-02	N/A
CD4% easy count kit		
CD4% easy count kit to count absolute CD4+ T-lymphocytes and CD4 percentages	05-8405 05-8411	100 tests
Vial containing CD4 mAb PE	05-8405-01 05-8411-01	N/A
Vial containing CD45 mAb PE-Cy5	05-8405-02 05-8411-02	N/A
Bottle containing Buffer 1	05-8405-03 05-8411-03	N/A
Bottle containing Buffer 2	05-8405-04 05-8411-04	N/A
Others		
Count Check Beads green Fluorescent Bead controls for system check (correct volume pipetting)	05-4026	50 tests
Cleaning Solution	04-4017	250 ml
Sheath Fluid	04-4016	5 L, including tab
Decontamination Solution	04-4018	250 ml
Hypochlorite Solution	04-4019	250 ml

Items required but not provided

Item Description	Product code
Sample Tubes 3.5 ml	04-2000
A verified pipette 20 µl fix and pipette tips	please refer to manufacturers/ distributors of pipettes and pipette tips
A verified pipette 100 – 1000 µl variable and pipette tips	please refer to manufacturers/ distributors of pipettes and pipette tips
A verified pipette 10 µl fix and pipette tips (for CD4% assay)	please refer to manufacturers/ distributors of pipettes and pipette tips
Venous blood collection system with EDTA as an anticoagulant	please refer to manufacturers/ distributors of blood collection systems
Stopwatch	N/A

Storage

Store the antibody and buffer reagents in the dark at 2°C to 8°C. Do not freeze or expose the reagents to elevated temperatures and keep them away from direct sunlight.
Store Sheath Fluid (Ref. No. 04-4016) at 18°C -30 °C.

Shelf-life upon manufacture

16 months.

Under the storage conditions mentioned above, both the CD4 easy count kit and the CD4% easy count kit will be stable until the expiration date printed on the kit label.

Warnings/limitations

For a complete list of warnings and precautions, refer to the current version of the manufacturer's instructions for use (IFU) and the Safety Data Sheet.

Prioritisation for prequalification

Based on the established eligibility criteria, CyFlow Counter System with CD4 easy count kit and CD4% easy count kit was given priority for WHO prequalification assessment.

Dossier assessment

Sysmex Partec GmbH submitted a product dossier for CyFlow Counter System with CD4 easy count kit and CD4% easy count kit as per the "*Instructions for compilation of a product dossier*" (PQDx_018 v1). The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

The manufacturer's responses to the nonconformities found during dossier screening and assessment findings were accepted on 24 May 2018.

Based on the product dossier screening and assessment findings, the product dossier for CyFlow Counter System with CD4 easy count kit and CD4% easy count kit meets WHO prequalification requirements.

Manufacturing site inspection

A comprehensive inspection was performed at the sites of manufacture (Sysmex Partec GmbH, Arndtstr. 11 a-b, 02826 Görlitz, Germany and Exbio Praha a.s., Nad Safinou II 341, 252 50 Vestec, Czech Republic) of CyFlow Counter System with CD4 easy count kit and CD4% easy count kit in October 2021 as per the "*Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics*" (PQDx_014 v4). The inspection found that the manufacturers had an acceptable quality management system and good manufacturing practices that ensured the consistent manufacture of a product of good quality.

The manufacturers' responses to the nonconformities found at the time of the inspection were accepted on 12 May 2022.

Based on the site inspection, corrective action plan review, and commitments identified and subsequently closed, the quality management system for CyFlow Counter System with CD4 easy count kit and CD4% easy count kit meets WHO prequalification requirements.

Product performance evaluation

CyFlow Counter System with CD4 easy count kit and CD4% easy count kit was evaluated at the Institute of Tropical Medicine (ITM) Antwerp, Belgium, which is a WHO Evaluating Laboratory for CD4 enumeration between October 2017 and February 2018. The performance evaluation was conducted using the WHO evaluation protocol (PQDx_114), which was also approved by the in-country ethical review board in Belgium.

A total of 312 fresh venous whole blood specimens were used to study failure rates, reproducibility (intra-assay variation, inter-assay variation, inter-instrument variation,

instrument precision) and agreement with the BD FACSCalibur (BD Biosciences) as the reference method using an antibody panel including CD3/CD4/CD8/CD45 monoclonal antibodies (Multiset, BD Biosciences) with Trucount tubes (Becton Dickinson). Lastly, ease of use was assessed.

The acceptance criteria were as follows: specimen failure should be less than 10%. For reproducibility studies, a percentage coefficient of variation (%CV) should be less than 15% for CD4+ T cell counts of less than or equal to 200/µL and %CV should be less than 10% for CD4 counts of more than 200 cells/µL. Compared to the reference method, the bias should be less than 10%.

Specimen failure, which was defined as failure of the instrument to provide valid results, was found to be 2.2 % for venous whole blood specimens.

Testing of fresh specimens was conducted to assess the ability of the CyFlow Counter System with CD4 easy count kit and CD4% easy count kit to provide reproducible results. The overall CV was less than 5% for CD4 absolute counts and CD4%. Individual CV's per CD4 category were all below 5 % for CD4 T cells, well within the WHO acceptance criteria (<10% and less than15% for CD4 counts below 200 cells/µL).

The inter-instrument precision was generally below 5% for absolute CD4 counts and consistently below 5% for CD4%, well below the acceptance criteria of WHO.

For the intra-instrument precision, all blood specimens showed a %CV less than 5 % for both CD4 counts and CD4% on venous blood specimens.

The average inter-assay variability for whole blood was between 4.0 and 4.2% for CD4 %, while it was between 3.4 and 4.9% for absolute CD4 counts.

The inter-assay variability (day-to-day reproducibility) of the normal control was mostly less than 5% (one exception with 5.2%). The low controls were generally between 5-10%, which is normal for low CD4 counts. In comparison, the variability on FACS Calibur was less than 5%.

The inter-assay variability on Multi Check stabilised blood indicated that both CyFlow Counter instruments had a good inter-assay reproducibility as %CV on normal blood controls and was generally less than 5%. The %CV was higher for low controls, but this was expected as precision is decreasing with low counts (<200 CD4 cells/µL), but the %CV on low counts was generally smaller than 10% and, in two cases, less than 12%, well within WHO's acceptance criteria. In comparison, the %CV on FACSCalibur were less than 5% for normal controls and slightly higher for low controls (5-7%).

Carry-over of the CD4 easy count kit was 0.58%, while for the CD4% easy count kit, it was 0.92%.

Regarding the agreement with the reference method, both kits, CD4 easy count kit and CD4% easy count kit, had a clear tendency towards a negative bias compared to FACS Calibur using TruCount tubes. The relative bias was smaller than 10% for the CD4 easy count kit, thus within the WHO acceptance criteria. The relative bias of the CD4% easy count kit was -3,5%, -10,6% and -11,7% for <200, 200 – 500 and >500 cells/µL respectively. The reason why the CD4% easy count kit had a larger negative bias than the CD4 easy count kit is unclear.
When compared to a dual platform, both kits CD4 easy count kit and CD4% easy count kit also showed a clear tendency towards a negative bias as compared to the dual platform: FACS Calibur CD4 percentages and total lymphocyte counts from Abbott Cell Dyn Ruby haematology analyser. The relative negative bias was smaller than 10% for the CD4 easy count kit, thus within the acceptance criteria. The relative bias of the CD4% easy count kit was larger than -10% for the highest CD4 category.

Two technicians involved in the laboratory study assessed the practical use of the CyFlow Counter system. The instrument was considered easy to handle and relatively simple and straightforward to use. The start-up and closing down procedures were relatively short. The time to prepare and stain a blood sample is relatively short (15 min).

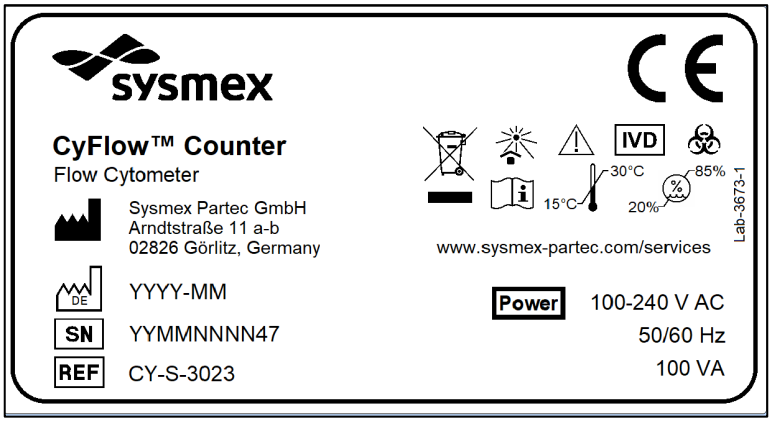
Labelling

1. **Labels**
2. **Instructions for use**

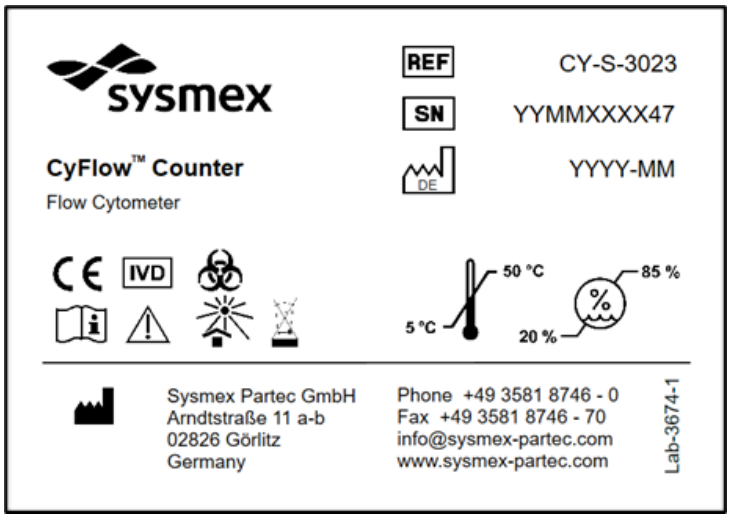
1. Labels

1.1 CyFlow™ Counter (CY-S-3023)

Back Plate Label CyFlow Counter



1.2. Shipping Carton Label CyFlow Counter

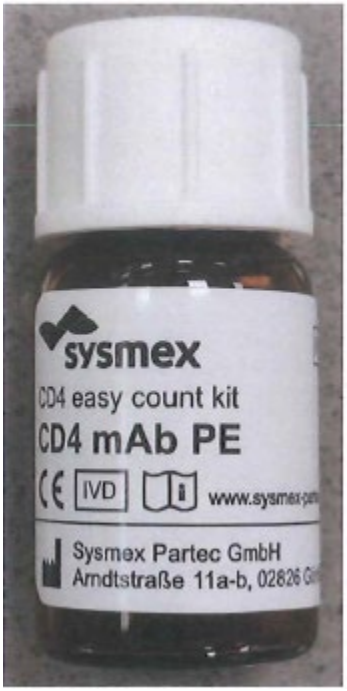


UDI Label

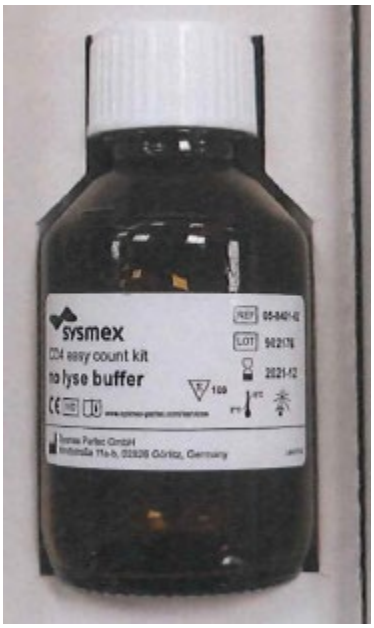
1.3 CD4 Easy Count Kit (05-8401)

1.3.1 Box Label

1.3.2 Bottle Label for CD4 mAb PE



1.3.3 Bottle Label for no lyse buffer



1.4. CD 4 Easy Count kit (05-8410)- Package labelling

1.4.1. Box label

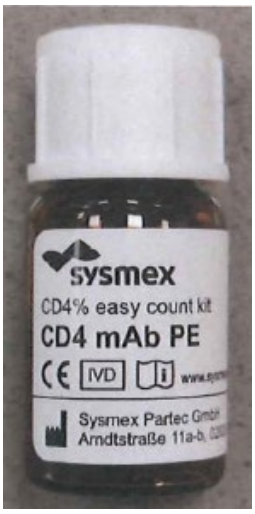
1.4.2 Bottle Label for CD4 mAb PE

1.4.3. Bottle Label for no lyse buffer

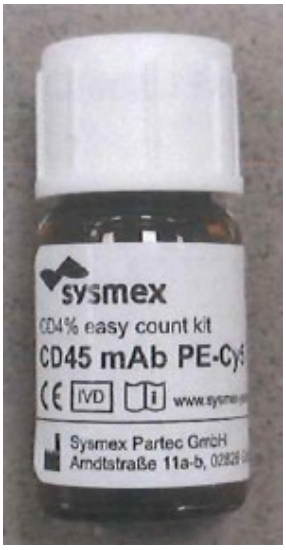
1.5 CD4% Easy Count Kit (05-8405)

1.5.1 Box Label

1.5.2 Bottle Label for CD4 mAb PE

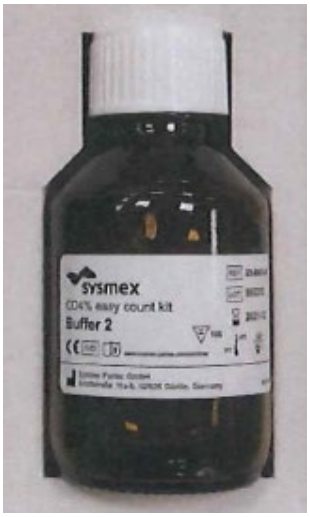


1.5.3 Bottle Label for CD45 mAb PE-Cy5



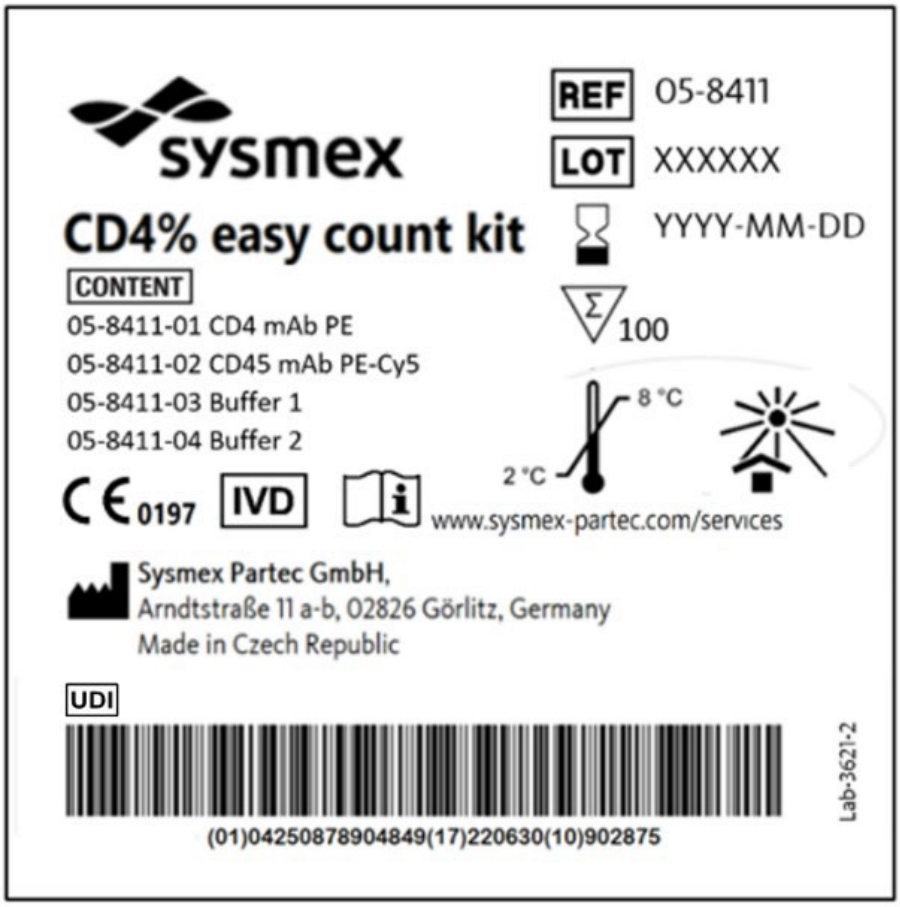
1.5.4 Bottle Label for Buffer 1

1.5.5 Bottle Label for Buffer 2



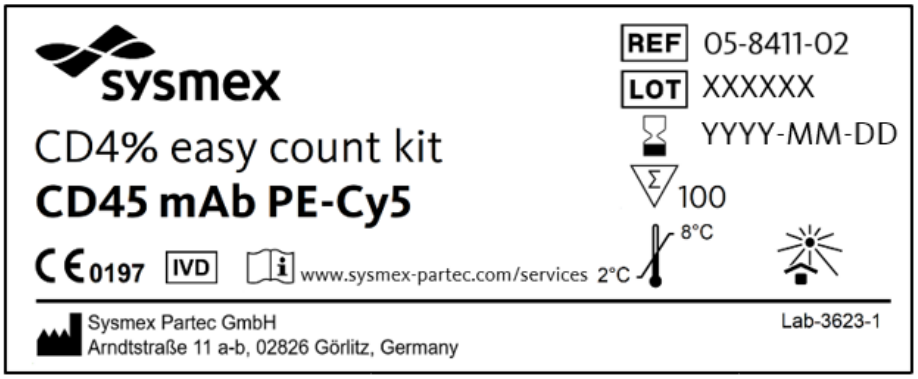
1.6 CD4% Easy Count Kit (05-8411)

1.6.1 Box Label

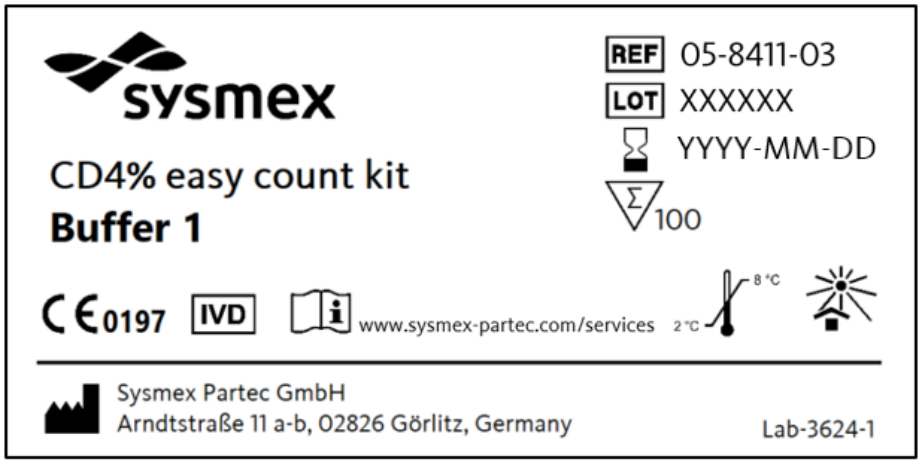


1.6.2 Bottle Label for CD4 mAb PE

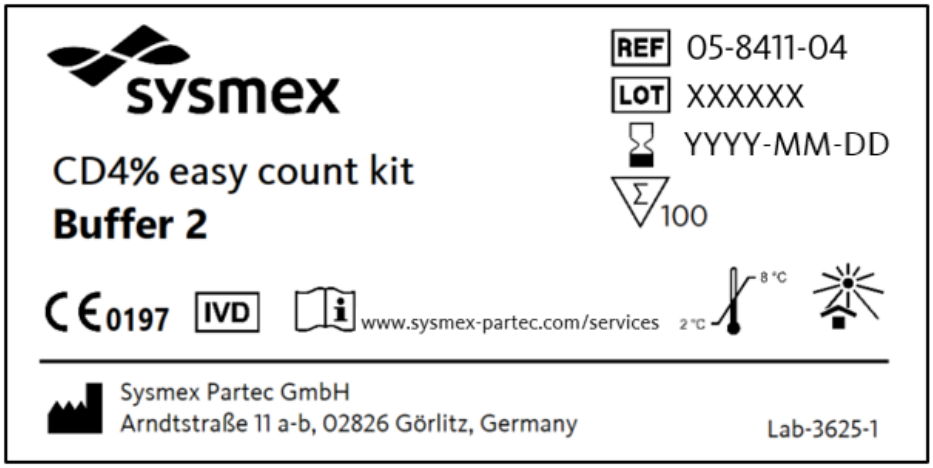
1.6.3 Bottle Label for CD45 mAb PE-Cy5



1.6.4 Bottle Label for Buffer 1

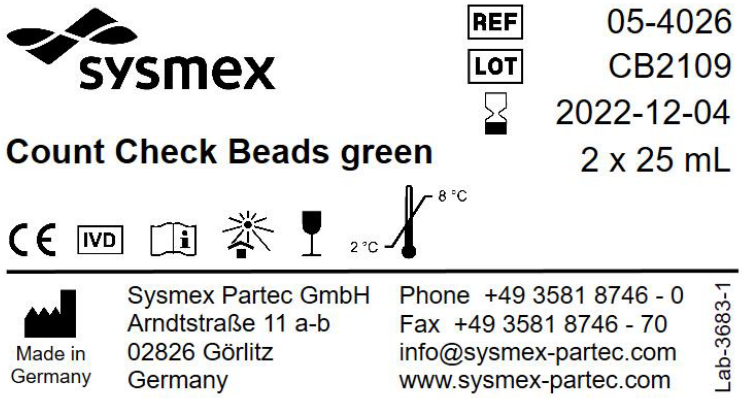


1.6.5 Bottle Label for Buffer 2

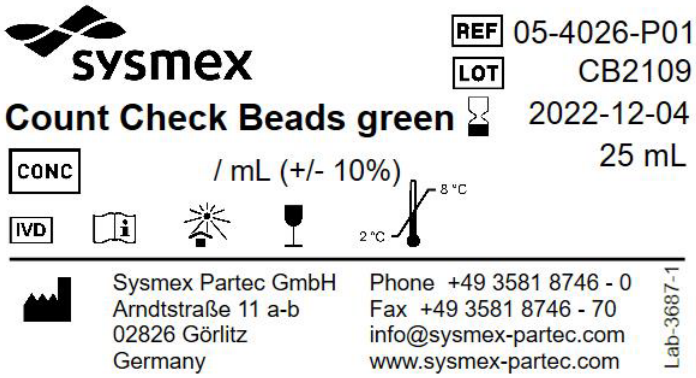


1.7 Count Check Beads green (05-4026)

1.7.1 Package Label



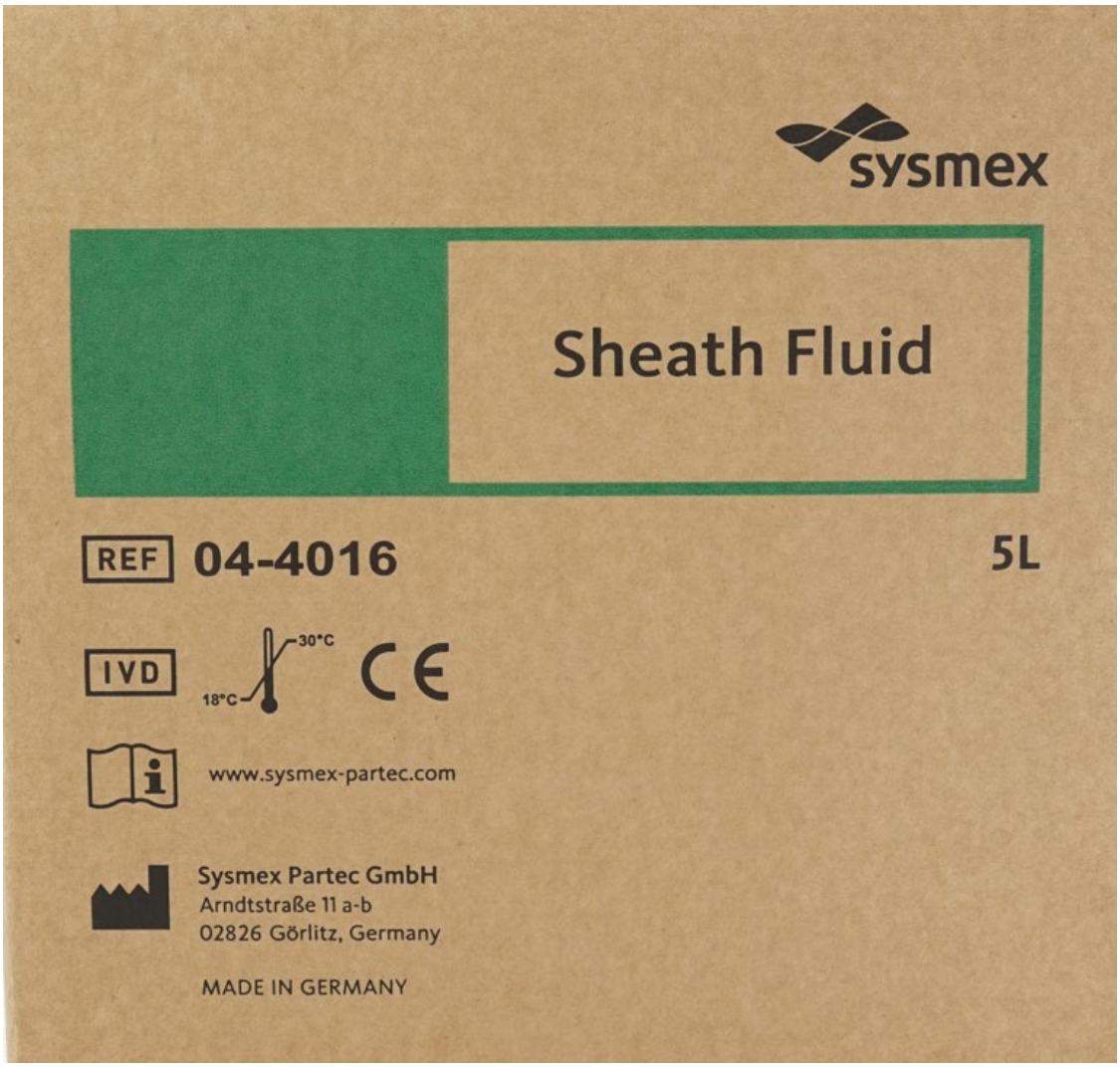
1.7.2 Bottle Label



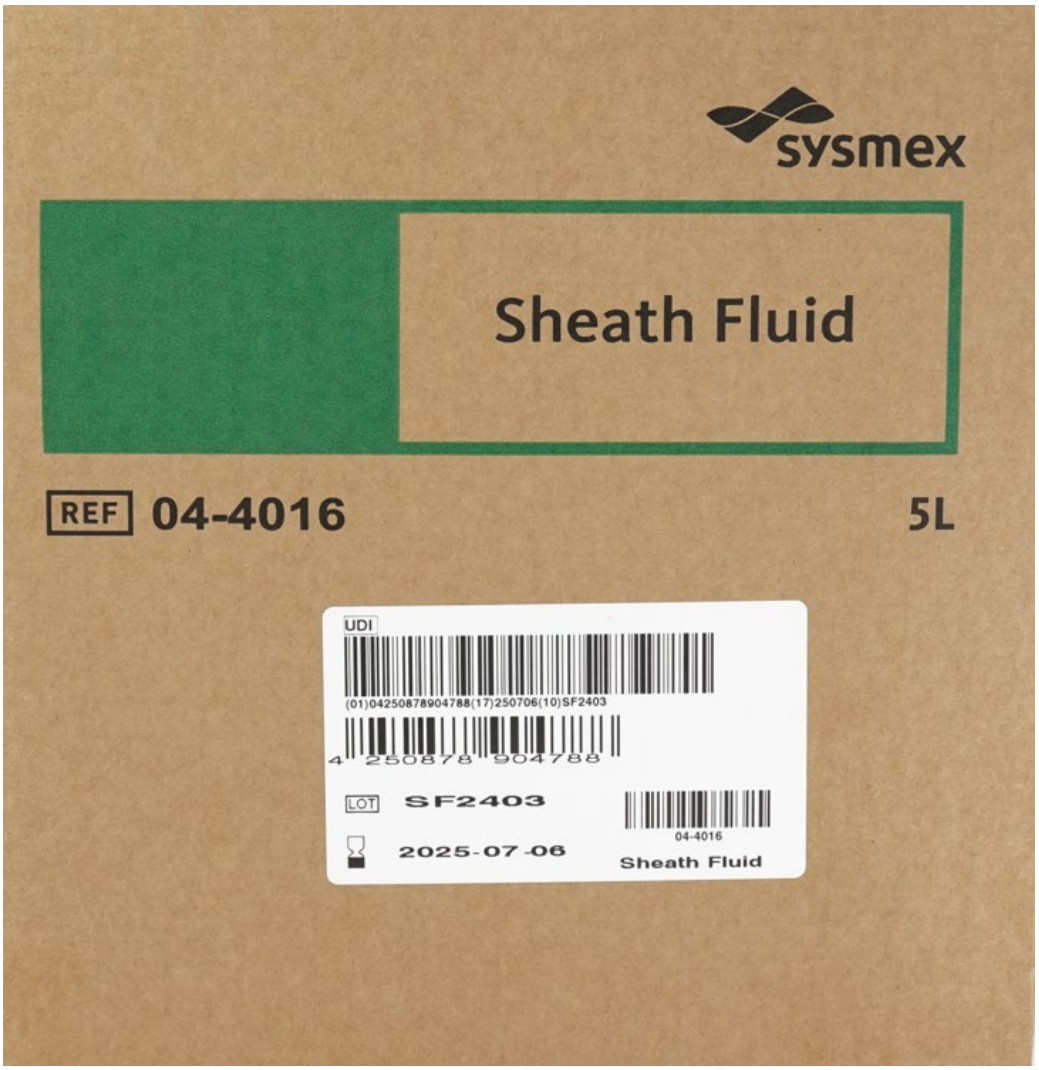
UDI Label

1.8 Sheath Fluid (04-4016)

1.8.1 Box Label 1



1.8.2 Box Label 2



1.8.3 Box Label 3

1.8.4 Box Label 4

IVD CE

sysmex

REF 04-4016 Instructions for use EN

1 Identification of the IVD reagent

Name	Sheath Fluid
Ref. No.	04-4016
UDI-DI	04250878904788
Content	5000 mL container incl. tap, ready to use

2 Intended Purpose

IVD For In Vitro Diagnostic Use.

Sheath Fluid is intended to ensure that the sample, fed to the Sysmex Partec clinical flow cytometer, will run under hydrodynamic focusing. Sample run, and Sheath Fluid consumption will be controlled via FCM software automatically. Sheath Fluid is a general laboratory accessory solution and does not provide any diagnostic information. Handling with Sheath Fluid is restricted to lab technicians and trained FCM operators.

3 Principle of the procedure

Sheath Fluid as an accessory solution for flow cytometry will be passed through the fluidic system of the flow cytometer via vacuum pressure. Once the Sheath Fluid is running at laminar flow, the sample flow will be injected into the center of the stream, at a slightly higher pressure. The principles of hydrodynamic focusing cause the cells to align, single file in the direction of flow.

For further information, please refer to the Instructions for Use (IFU) of your flow cytometer.

4 Storage and shelf life

4.1 Unopened product

Store at 18 - 30°C, do not freeze or expose to elevated temperatures. Under these storage conditions the reagent will be stable until the expiration date printed on its label. Do not use the reagent beyond its shelf life.

4.2 Product after first opening

Sheath Fluid will be stable at least 7 days after first use. Always use a new clean tap after the Sheath Fluid container was first opened. Close the tap after each use, to avoid contamination.

5 Components

5000 mL aqueous solution

6 Evidence of deterioration

Sheath Fluid is a clear liquid. Do not use Sheath Fluid after appearance of any kind of turbidity or contamination.

For questions regarding to the performance or quality of the product received, please contact your local Sysmex Representative.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user is located.

7 Precautions and warnings

Appropriate safety precautions and handling procedures must be applied in accordance with applicable laws and regulations.

Refer to the Safety Data Sheet (SDS) for a complete list of warnings and precautions.

8 Additional required equipment

Instrument:

Sysmex Partec clinical flow cytometer, such as the CyFlow™ Counter (Ref. No. CY-S-3023)

For further information, please refer to the instruction for use (IFU) of the flow cytometer.

Laboratory equipment:

A calibrated pipette and pipette tips

Sample tube(s) compliant to the flow cytometer

Personal protective equipment

9 Disposal

Disposal procedure should meet requirements of applicable local regulations.

10 Manufacturer

Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz
Germany
Phone +49 3581 8746 0
Fax +49 3581 8746 70
info@sysmex-partec.com
www.sysmex-partec.com

11 Symbols

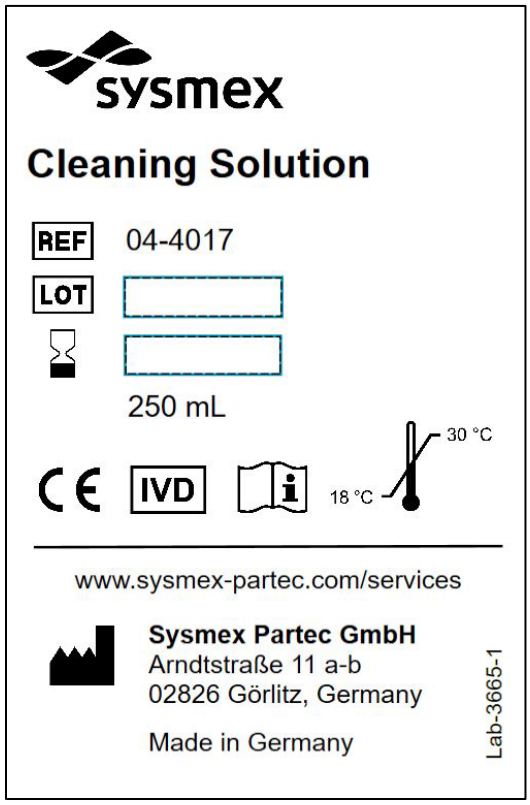
REF	Catalogue number		Manufacturer
LOT	Batch code		Use-by date
	Temperature limit		Keep dry
CE	CE-Mark		Stacking limit by number
	This way up		Use no hooks
	Consult instructions for use	UDI	Unique device identifier
IVD	In vitro diagnostic medical device		

12 Date of issue or revision

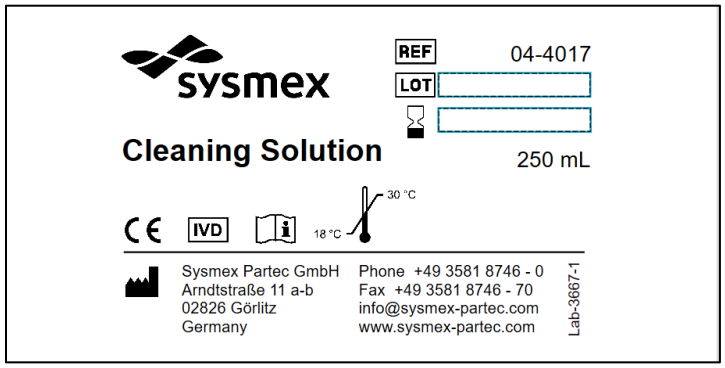
Rev.:	001	CN 2151
Rev. date:	30-09-2021	
Doc. No.:	Lab-3655-1	

1.9 Cleaning Solution (04-4017)

1.9.1 Package label

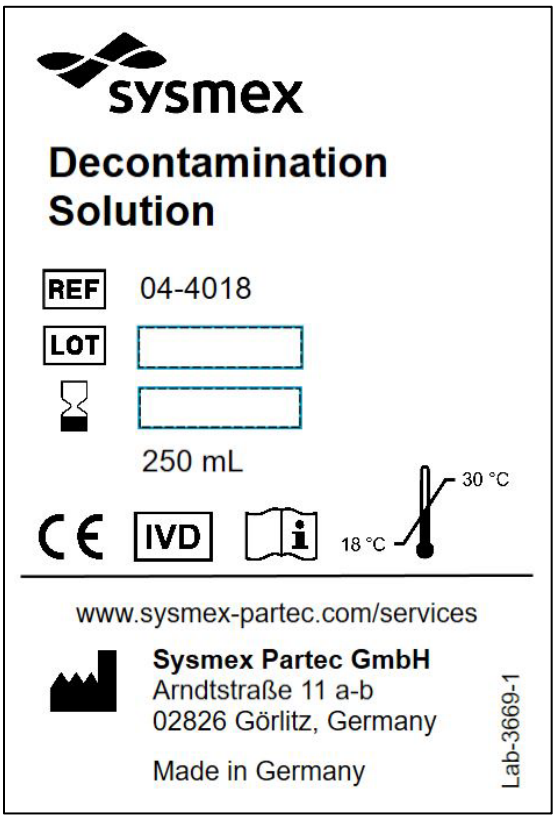


1.9.2 Bottle label

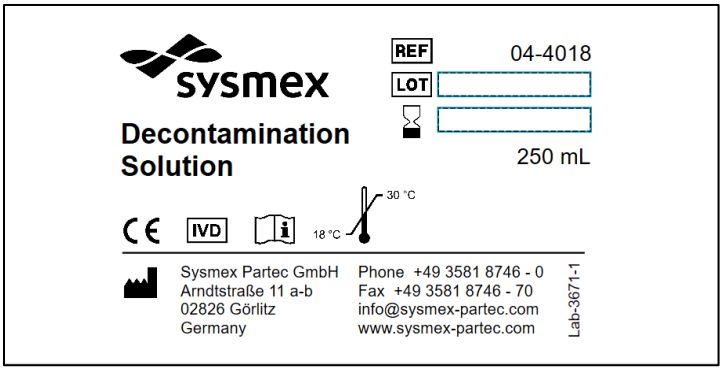


1.10 Decontamination Solution (04-4018)

1.10.1 Package label

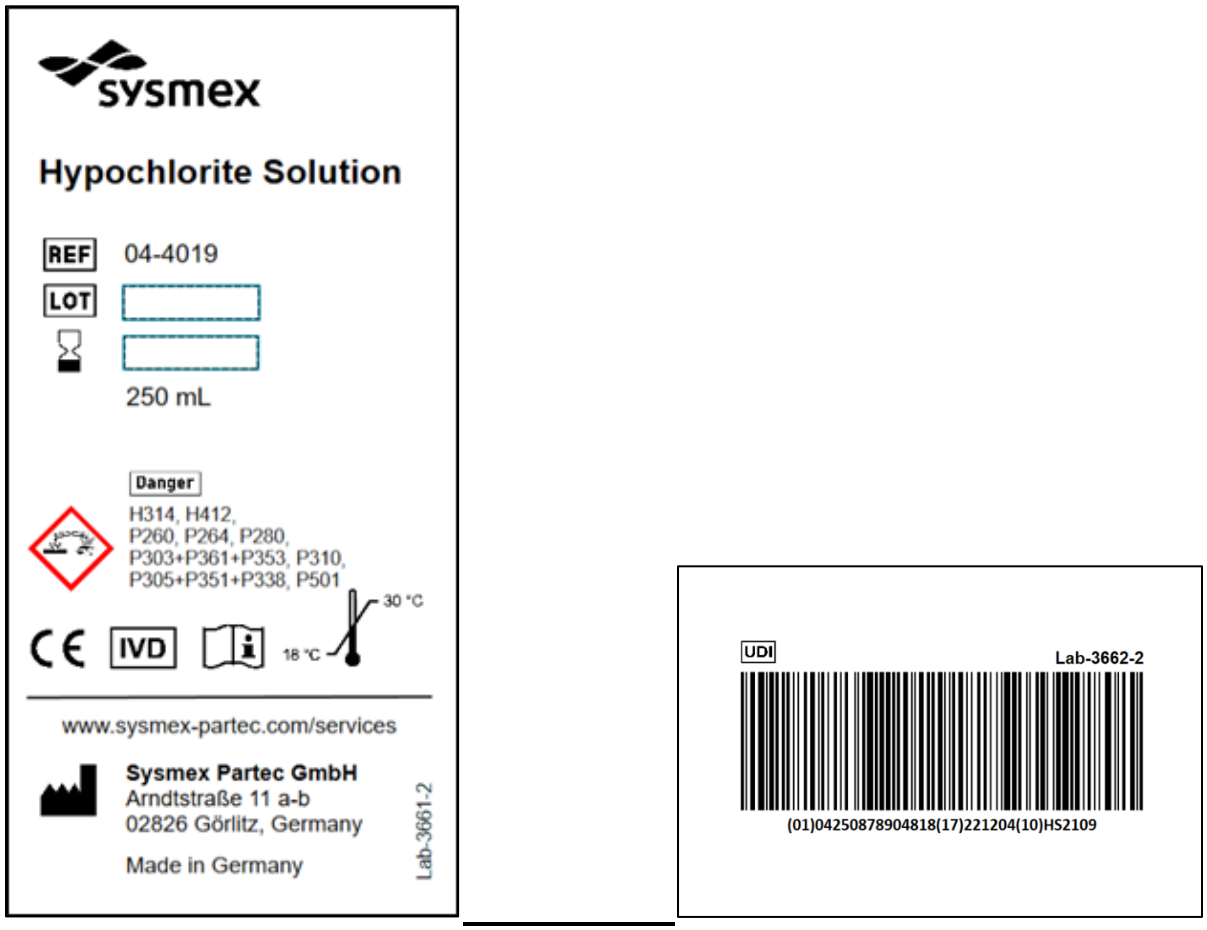


1.10.2 Bottle label

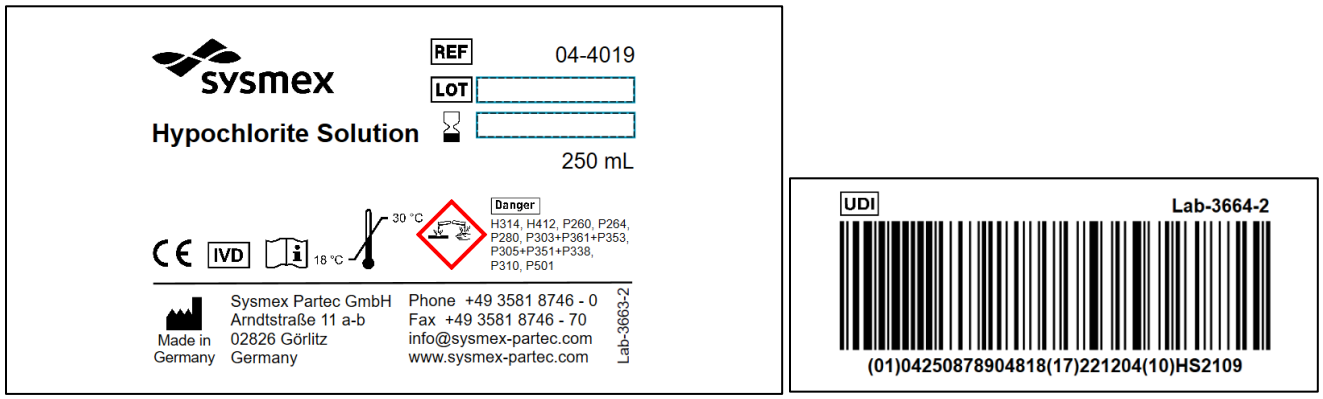


1.11 Hypochlorite Solution (04-4019)

1.11.1 Package label



1.11.2 Bottle label



2. Instructions for use[1]

[1] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

1.1 CD4 easy count kit (product code 05-8401)

IVD CE

Instructions for Use

Identification of the IVD reagent

Product name:

CD4 easy count kit

REF 05-8401

Content:

Vial containing CD4 mAb PE — REF 05-8401-01
Bottle containing no lyse buffer — REF 05-8401-02

1 Specification

Specificity	Human CD4
Isotype	IgG1
Content	100 tests
Fluorochrome	PE
λ excitation (nm)	532 / 488
Emission maximum (nm)	578

2 Intended Use

IVD For In Vitro Diagnostic Use.

The CD4 easy count kit is a manual, two component, quantitative IVD test for subpopulation labelling of lymphocytes in adult venous EDTA whole blood, and subsequent enumeration with the Sysmex Partec CyFlow™ Counter IVD flow cytometer. The CD4 T cell concentration of blood samples is a useful indicator for the initiation or follow-up of treatment for HIV positive patients in conjunction with other laboratory and clinical findings.
The test is intended to be performed by trained healthcare professionals.

3 Principle of the examination method

An aliquot of an EDTA whole-blood sample is mixed with the antibody (CD4) conjugated to the fluorochrome in a 1:1 ratio. After a fixed incubation time, the buffer is added and the sample is ready for analysis on the CyFlow™ Counter flow cytometer.
The light source excites the fluorescent dye linked with the stained cell and the emitted light is detected while a certain volume of blood sample is running through the instrument.
The integrated software calculates the concentration of the dedicated cell populations.
For further information, please refer to the IFU of CyFlow™ Counter (CY-S-3022IFUEN).

4 Storage and shelf life after first opening

1. Storage
Store the antibody and buffer reagents at 2 - 8°C in the dark. Do not freeze or expose the reagents to elevated temperatures and keep them away from direct sunlight.
2. Shelf life after first opening:
Always close the bottle after use and use new pipette tips each time the reagent is sampled to avoid contamination.
Keep the antibody and buffer reagent at 2 - 8°C in the dark. Do not freeze or expose the reagents to elevated temperatures and keep them away from direct sunlight.
Under these storage conditions, the CD4 easy count kit will be stable until the expiration date printed on the kit label.

5 Components

CD4 mAb PE is a murine monoclonal antibody supplied in PBS buffer with 0.2% BSA and 0.09% sodium azide. No lyse buffer is a PBS-based solution containing 0.09% sodium azide.

6 Evidence of deterioration

The antibody and buffer solutions are clear liquids. Do not use the reagents if there is the appearance of any kind of turbidity or contamination.
For questions regarding the performance or quality of the product received, please contact your local Sysmex representative.

7 Precaution and warnings

Reagents contain 14 mM sodium azide as a preservative. The low concentration of sodium azide does not require hazard labelling, but the normal safety precautions for the handling of chemicals must be observed. Refer to the Safety Data Sheet (SDS) for a complete list of warnings and precautions.

8 Additional required equipment

Instrument requirement:	CyFlow™ Counter (REF No.: CY-S-3022)
Required apparatus:	Sample Tubes 3.5 ml (REF No.: 04-2000) A verified pipette 20 μl and pipette tips A verified pipette 100 – 1000 μl and pipette tips Venous blood collection system with EDTA as anticoagulant Stop watch

9 Reagent preparation

CD4 mAb PE (REF No.: 05-8401-01), the antibody reagent is ready to use.
No lyse buffer (REF No.: 05-8401-02), the buffer solution is ready to use.

10 Primary sample collection, handling and storage

WARNING ⚠ *All biological specimens and material are considered as biohazards and should be handled as if capable of transmitting infection. Appropriate safety precautions and handling procedures must be applied in accordance with applicable laws and regulations.*

- The blood sample must be a venous, whole-blood sample, collected with a blood collection system which contains EDTA as anticoagulant.
- The blood sample must be transported in a dark container, protected from light and not exposed to elevated temperatures. A suitable transport container is, for example, a Styrofoam box with cooling material. Best transport conditions would be below 25 °C if transported will not take more than 6 hours after donation. For transport and storage time more than 6 hors, blood must be kept at 2 - 8 °C.
- Do not freeze and thaw the blood sample.
- For best conditions, the blood sample should be fresh, i.e. not more than six hours should have elapsed between sample collection and analysis.
- Blood samples can be used up to 24 hours after drawing if they are stored in the fridge at 2 – 8 °C.
- Before use, the sample tubes containing the blood must be inverted gently 8 to 10 times.
- Do not use clotted blood samples.

11 Examination procedure

NOTICE *Reverse pipetting is critical to accuracy especially when dispensing viscous and very small sample volumes. For whole blood pipetting, we recommend using a calibrated electronic pipette which is preprogrammed to operate in the reverse pipetting mode.*
If an electronic pipette is not available, follow these instructions for manual reverse pipetting:
- *Depress the operating button to the second stop. Let the operating button move up completely, excess sample is drawn up into the tip.*
- *Depress the operating button to the first stop to expel a precise volume of blood, leaving excess sample in the tip.*
- *Discard pipette tip with excess blood sample in the tip into medical waste container.*

Please note, you must not use reverse pipetting for antibody or buffer solutions.

11.1 Staining

- Invert the blood sample in the EDTA blood collection tube gently 8 to 10 times.
- Pipette 20 μl EDTA whole blood sample to the bottom of a sample tube, avoid leaving a trail of blood on the inner tube wall from pipette tip. Discard the pipette tip.
- Add 20 μl CD4 mAb PE directly into the blood sample and mix it gently, avoid leaving a trail of blood on the inner tube wall from pipette tip. Discard the pipette tip.
- The sample should not be vortexed at this stage of preparation.
- Incubate the mixture for 15 minutes at room temperature in the dark.
- Add 800 μl no lyse buffer and vortex briefly, or tap tube gently in order to mix the sample. Discard the pipette tip.
- Vortex briefly, or tab tube gently before analysing with the CyFlow™ Counter.

NOTICE *The stained blood sample must be analysed within 2 hours after adding the no lyse buffer.*

11.2 Sample analysis

Please refer to the IFU of the CyFlow™ Counter (CY-S-3022IFUEN) for how to operate the instrument before analysing the samples. The start-up procedures and the internal quality control procedure must be successfully completed before sample analysis.
- Choose and load the configuration for CD4 measurement from the ‚menu bar of CyFlow™ Counter.
- Insert the sample tube with the prepared blood sample into the sample port.
- Start measurement.
- After sample run, the instrument stops and cleans automatically.
- Remove and dispose of blood sample in accordance with local laboratory biohazard safety procedures.

Do not start a second measurement with the same sample tube. If a duplicate or repeat measurement is required, a second sample tube should be prepared as per 11.1.

11.3 Data acquisition and analysis

Data analysis with the CyFlow™ Counter is only possible if the internal quality control of the instrument was successful.
Please refer to the IFU of the CyFlow™ Counter (CY-S-3022IFUEN) for proper start-up procedure and maintenance of the device, if necessary.
The CD4 T-cell counting result will be displayed on the screen.
If the histogram does not show a clear peak of the CD4 positive stained T-cells, the sample preparation and measurement must be repeated.
Results will be incorrect if gate positioning is not done precisely.
Be careful not to gate monocytes as CD4 positive stained cells if CD4 concentration is extremely low in a patient blood sample.

12 Calculation of examination results

Calculation of the cell concentration is part of the software-based data analysis, which provides the result in CD4 cells per μl blood sample after each measurement.
For further information, please refer to the IFU of the CyFlow™ Counter (CY-S-3022IFUEN).

13 Interpretation of results

Human Immunodeficiency Virus (HIV) is one of the main reasons for CD4 cell depletion, which debilates the immune system. Results should be interpreted by physicians, in conjunction with the locally applicable HIV treatment guidelines.

14 Control procedure

Appropriate quality control should be performed according to local and national regulations. A variety of stabilized blood samples are available on the market, only a few are suitable when using certain flow cytometers.If the acceptance criterion of the individual control material is not met do not proceed with patient specimen testing and contact your local Sysmex representative.
For further information about the use of adequate control material, please contact your local Sysmex representative.

15 Performance characteristics

15.1 Specificity

The mouse monoclonal antibody MEM-241 recognizes the human CD4 antigen, a transmembrane glycoprotein (55 kDa) of the immunoglobulin supergene family, present on a subset of T-lymphocytes (“helper/inducer” T-cells) and expressed at a lower level on monocytes, tissue macrophages and granulocytes. HCDM (former HLDA VIII) Meeting, May 2006, Québec, Canada; WS Code M241 Detection capability. The evaluation for Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) was carried out according to the specifications in the guideline CLSI EP17-A2.
LoB: ≤50% of LoD
LoD: ≤20 CD4 cells/μl
LoQ: ≤40 CD4 cells/μl

15.2 Repeatability

Intra- and inter-assay variation:
CV values are less than 10% for CD4 values of 200 CD4 T-cells/μl or above.
CV values are less than 15% for CD4 absolute values below 200 CD4 T-cells/μl.

15.3 Linearity

The measuring range of the assay and linearity of the measurement are valid over the range:
CD4 absolute: 40-2500 CD4 T-cells/μl

15.4 Trueness

EDTA whole blood specimen were stained with CD4 easy count kit and analysed on the CyFlow™ Counter. CD4 absolute values were compared with results from a BD FACSCalibur using BD Tritest CD3/CD4/CD45 and BD Trucount tubes and showed a bias of -15,13% between both methods.

16 Limitations

CAUTION ⚠ *Sysmex Partec CD4 easy count kits (05-8401) have not been validated for use with pediatric and adolescent patients.*

CAUTION ⚠ *Patient measurements of CD4 and CD4% made using Sysmex Partec assays should not be used independently with other manufacturer’s methods of determining CD4 and CD4%. Use the assays only with the Sysmex Partec instruments. Values obtained using other equipment or assays may not be interchangeable.*

Different endogenous and exogenous substances were tested regarding the CLSI EP07-A2 guideline for possible interfering effects which could have an influence to the blood sample analysis. Finally, ≥ 18% haemolysis (induced by using the protocol recommended in the CLSI EP07-A2, Appendix G, G1 - Osmotic Shock Procedure) was observed to show an interfering effect. For that reason haemolyzed samples should be rejected.
For further questions regarding the performance of the product, please contact your local Sysmex representative.

17 Literature references

1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for Determining Percentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Contact of manufacturer

Manufacturer
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Germany
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Version number of IFU and date of issue

Revision: Rev-007_10-09-2020_CN 1563
Issued by: Sysmex Partec GmbH

Safety Data Sheet for this product is available at www.sysmex-partec.com/services.

Benutzerhandbuch

Identifikation des IVD-Reagens

Produktname:

CD4 easy count kit

REF 05-8401

Inhalt:

Ampulle mit CD4 mAb PE — REF 05-8401-01
Flasche mit No-Lyse-Puffer — REF 05-8401-02

1 Spezifikation

Spezifität	Humanes CD4
Isotyp	IgG1
Inhalt	100 Tests
Fluorochrom	PE
λ Anregung (nm)	532 / 488
Emissionsmaximum (nm)	578

2 Zweckbestimmung

IVD In-vitro-Diagnostikum.

Das CD4 easy count kit ist ein manueller, quantitativer 2-Komponenten-In-vitro-Test für die Markierung von Lymphozyten-Untergruppen in venösen EDTA-Vollblutproben von Erwachsenen und deren anschließender Auszählung mit dem Sysmex Partec IVD-Flowzytometer CyFlow™ Counter. Die Konzentration der CD4-positiven T-Helferzellen in Blutproben ist in Verbindung mit anderen Laborwerten und klinischen Befunden ein nützlicher Indikator für die Einleitung der Behandlung HIVpositiver Patienten und die Nachsorge.
Der Test ist nur von geschulten medizinischen Fachkräften durchzuführen.

3 Prinzip des Untersuchungsverfahrens

Ein Aliquot einer EDTA-Vollblutprobe wird mit dem an das Fluorochrom konjugierten Antikörper (CD4) in einem Verhältnis von 1:1 gemischt, Nach einer bestimmten Inkubationszeit wird der Puffer hinzugegeben; nun kann die Analyse der Probe in einem CyFlow™ Counter-Flowzytometer erfolgen. Die Lichtquelle regt den Fluoreszenzfarbstoff an, mit dem die Zellen markiert wurden, und das emittierte Licht wird, während ein bestimmtes Volumen der Blutprobe das Instrument durchläuft, detektiert. Die Konzentration der spezifischen Zellpopulation wird von der integrierten Software berechnet. Weitere Informationen entnehmen Sie bitte dem Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFUEN).

4 Lagerung und Haltbarkeitsdauer nach dem ersten Öffnen

1. Lagerung:
Das Antikörper- und Pufferreagens bei 2 – 8 °C im Dunkeln aufbewahren. Nicht einfrieren oder erhöhten Temperaturen aussetzen. Vor direkter Sonneneinstrahlung schützen.
2. Haltbarkeitsdauer nach dem ersten Öffnen:
Die Flasche nach jedem Gebrauch wieder verschließen und für jede weitere Entnahme des Reagens neue Pipettenspitzen verwenden, um eine Verunreinigung zu vermeiden.
Das Antikörper- und Pufferreagens bei 2 – 8 °C im Dunkeln aufbewahren. Nicht einfrieren oder erhöhten Temperaturen aussetzen. Vor direkter Sonneneinstrahlung schützen.
Unter Einhaltung der oben genannten Lagerbedingungen ist das CD4 easy count kit bis zum Verfallsdatum auf dem Etikett des Kits stabil.

5 Bestandteile

CD4 mAb PE ist ein muriner monoklonaler Antikörper in PBS-Puffer mit 0,2 % BSA und 0,09 % Natriumazid.
No-Lyse-Puffer ist eine Lösung auf PBS-Basis, die 0,09 % Natriumazid enthält.

6 Anzeichen von Verderb

Die Antikörper- und Pufferlösungen sind klare Flüssigkeiten. Die Reagenzien nicht verwenden, falls Trübungen oder Verunreinigungen auftreten.
Bei Fragen zur Leistung oder Qualität des Produkts wenden Sie sich bitte an Ihre nächstgelegene Sysmex-Vertretung.

7 Vorsichtsmaßnahmen und Warnhinweise

Die Reagenzien enthalten 14 mM Natriumazid als Konservierungsstoff. Die geringe Konzentration von Natriumazid erfordert keine Gefahrenkennzeichnung, jedoch sind die beim Umgang mit Chemikalien üblichen Vorsichtsmaßregeln zu beachten. Beachten Sie das Sicherheitsdatenblatt (SDB) für eine vollständige Liste von Warnhinweise und Vorsichtsmaßnahmen.

8 Zusätzlich erforderliche Ausrüstung

Erforderliche Instrumente:	CyFlow™ Counter (REF No.: CY-S-3022)
Benötigte Ausrüstung:	Sample Tubes 3.5 ml (REF No.: 04-2000) Eine verifizierte 20-μl-Pipette und Pipettenspitzen Eine verifizierte 100- bis 1000-μl-Pipette und Pipettenspitzen System zur venösen Blutentnahme mit EDTA als Antikoagulans Stoppuhr

9 Reagensvorbereitung

CD4 mAb PE (REF No.: 05-8401-01), das Antikörperreagens ist gebrauchsfertig.
No-Lyse-Puffer (REF No.: 05-8401-02), die Pufferlösung ist gebrauchsfertig.

10 Entnahme, Handhabung und Lagerung der Primärprobe

WARNUNG ⚠ *Alle biologischen Proben und Materialien sind als gefährliches biologisches Material und als potenziell infektiös zu behandeln. Es sind angemessene Sicherheitsvorkehrungen und Verfahren zur Handhabung gemäß den geltenden Gesetzen und Vorschriften anzuwenden.*

- Bei der Blutprobe muss es sich um eine venöse Vollblut-Probe handeln, die mithilfe eines Blutentnahmesystems entnommen wurde, das EDTA als Antikoagulans verwendet.
- Die Blutprobe ist in einem dunklen, vor Licht geschützten Behälter zu transportieren und darf nicht erhöhten Temperaturen ausgesetzt werden. Ein geeigneter Transportbehälter ist z. B. eine Styroporbox mit Kühlung. Die besten Transportbedingungen würden unter 25 °C liegen, wenn der Transport nicht länger als 6 Stunden nach der Spende dauern würde. Für eine Transport- und Lagerzeit von mehr als 6 Stunden muss das Blut bei 2 - 8 °C aufbewahrt werden.
- Die Blutprobe nicht einfrieren und wieder auftauen.
- Optimale Bedingungen sind bei Verwendung einer frischen Blutprobe gegeben, d. h. es sollten nicht mehr als sechs Stunden zwischen der Blutentnahme und der Analyse liegen.
- Bei Lagerung im Kühlschrank bei 2–8 °C können Blutproben bis zu 24 Stunden nach der Entnahme verwendet werden.
- Vor der Anwendung die Probenröhrchen 8- bis 10-mal vorsichtig umdrehen.
- Keine geronnenen Blutproben verwenden.

11 Untersuchungsverfahren

HINWEIS *Reverses Pipettieren ist entscheidend für die Genauigkeit, insbesondere bei der Abgabe viskoser und sehr kleiner Probenmengen. Für das Pipettieren von Vollblut empfehlen wir die Verwendung einer kalibrierten elektronischen Pipette, die für den Betrieb im reversen Pipettiermodus vorprogrammiert ist.*
Steht keine elektronische Pipette zur Verfügung, führen Sie das reverse Pipettieren gemäß den folgenden Anweisungen manuell durch:
- *Bedienknopf bis zum zweiten Anschlag herunterdrücken. Bedienknopf vollständig zurückgleiten lassen. Es wird eine größere Menge Probe in die Spitze aufgezogen.*
- *Bedienknopf bis zum ersten Anschlag herunterdrücken, um ein genaues Blutvolumen abzugeben. Anschließend befindet sich noch ein Rest der Probe in der Spitze.*
- *Pipettenspitze mit dem verbliebenen Rest der Blutprobe in einen Behälter für medizinische Abfälle entsorgen.*

Beachten Sie bitte, dass reverses Pipettieren nicht für Antikörper- oder Pufferlösungen eingesetzt werden darf.

11.1 Markierung

- Die Blutprobe im EDTA-Blutentnahmeröhrchen 8- bis 10-mal vorsichtig umdrehen.
- 20 μl EDTA-Vollblutprobe auf den Boden eines Probenröhrchens pipettieren. Dabei keine Blutspuren von der Pipettenspitze auf der Röhrcheninnenwand hinterlassen. Entsorgen Sie die Pipettenspitze.
- 20 μl CD4 mAb PE direkt in die Blutprobe geben und vorsichtig mischen. Dabei keine Blutspuren von der Pipettenspitze auf der Röhrcheninnenwand hinterlassen. Entsorgen Sie die Pipettenspitze.
- Probe zu diesem Zeitpunkt nicht vortexen.
- Inkubation für 15 Minuten bei 15-30 °C im Dunkeln.
- 800 μl No-Lyse-Puffer hinzugeben und kurz vortexen oder das Röhrchen sanft aufklopfen, um die Probe zu mischen. Entsorgen Sie die Pipettenspitze.
- Vor der Analyse mit dem CyFlow™ Counter kurz vortexen oder das Röhrchen sanft aufklopfen.

HINWEIS *Nach der Zugabe von No-Lyse-Puffer müssen die Proben innerhalb von 2 Stunden analysiert werden.*

11.2 Probenanalyse

Bitte vor der Probenanalyse das Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFUEN) für die Verwendung des Geräts beachten. Vor der Probenanalyse müssen die Verfahren zur Inbetriebnahme und internen Qualitätskontrolle erfolgreich abgeschlossen worden sein.
- Wählen und laden Sie die Konfiguration für die CD4-Messung aus der Menüleiste des CyFlow™ Counter.
- Das Probenröhrchen mit dem aufbereiteten Blut in den Probenport setzen.
- Die Messung starten.
- Nach dem Probendurchlauf stoppt das Instrument und wird automatisch gereinigt.
- Die Blutprobe entnehmen und gemäß den örtlichen Sicherheitsvorschriften in Bezug auf biogefährliche Materialien in Labors entsorgen.

Keine erneute Messung mit demselben Probenröhrchen durchführen. Wenn eine Zweifachmessung oder eine Wiederholung der Messung erforderlich ist, muss ein zweites Probenröhrchen wie unter 11.1 beschrieben vorbereitet werden.

11.3 Datenerfassung und -analyse

Die Datenanalyse kann erst erfolgen, wenn die interne Qualitätskontrolle des CyFlow™ Counter erfolgreich abgeschlossen wurde.
Im Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFUEN) finden Sie eine Beschreibung der korrekten Inbetriebnahme und Wartung des Geräts.
Die CD4-T-Zellzahl wird auf dem Bildschirm angezeigt. Zeigt das Histogramm keine eindeutige Erhöhung der markierten CD4-positiven T-Zellen an, muss die Probenvorbereitung und -messung wiederholt werden.
Es kommt zu falschen Ergebnissen, wenn das Gaten nicht korrekt durchgeführt wird.
Achten Sie darauf, Monozyten nicht als markierte CD4-positive Zellen zu gaten, wenn die CD4-Konzentration der Patientenblutprobe sehr niedrig ist.

12 Berechnung der Untersuchungsergebnisse

Die Berechnung der Zellkonzentration erfolgt im Rahmen der Software-basierten Datenanalyse. Die Ergebnisse werden nach jeder Messung als CD4-Zellen pro μl Blutprobe dargestellt.
Weitere Informationen entnehmen Sie bitte dem Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFUEN).

13 Interpretation der Ergebnisse

Das Humane Immundefizienzvirus (HIV) ist einer der Hauptgründe für die CD4-Zelldepletion, die das Immunsystem beeinträchtigt. Die Ergebnisse sollten von Ärzten in Verbindung mit lokal anwendbaren HIV-Behandlungsrichtlinien interpretiert werden.

14 Kontrollverfahren

Es sollte eine geeignete Qualitätskontrolle gemäß den geltenden nationalen Laborvorschriften durchgeführt werden.
Im Handel ist ein breites Angebot an stabilisierten Blutproben erhältlich, jedoch sind bei Verwendung bestimmter Flowzytometer nur wenige geeignet.
Wenn das Annahmekriterium des betreffenden Kontrollmaterials nicht erfüllt ist, die Patientenprobe nicht analysieren. Wenden Sie sich in diesem Fall an Ihre nächstgelegene Sysmex-Vertretung.
Weitere Informationen über die Verwendung geeigneten Kontrollmaterials können Sie ebenfalls bei Ihrer nächstgelegenen Sysmex-Vertretung erhalten.

15 Leistungsmerkmale

15.1 Spezifität

Der monoklonale Maus-Antikörper MEM-241 erkennt das humane CD4-Antigen, ein transmembranes Glykoprotein (55 kDa) der Immunglobulin-Superfamilie, das auf einer Untergruppe von T-Lymphozyten (T-Helferzellen) exprimiert wird, sowie in geringerem Maße auf Monozyten, Gewebsmakrophagen und Granulozyten. HCDM (ehemals HLDA VIII) Meeting, Mai 2006, Québec, Kanada; WS Code M241

15.2 Nachweisfähigkeit

Die Leerwert-Obergrenze (Limit of Blank, LoB), die Nachweisgrenze (Limit of Detection, LoD) und die Quantifizierungsgrenze (Limit of Quantitation, LoQ) wurden gemäß den Spezifikationen der Richtlinie CLSI EP17-A2 ermittelt.
LoB: ≤ 50 % der LoD
LoD: ≤ 20 CD4-Zellen/μl
LoQ: ≤ 40 CD4-Zellen/μl

15.3 Reproduzierbarkeit

Intra- und Inter-Assay-Varianz:
Die CV-Werte für CD4-Werte ≥ 200 CD4-T-Zellen/μl liegen unter 10 %.
Die CV-Werte für absolute CD4-Werte < 200 CD4-T-Zellen/μl liegen unter 15 %.

15.4 Linearität

Der Messbereich des Assays und die Linearität der Messungen gelten für den folgenden Bereich:
Absoluter CD4-Wert: 40–2500 CD4-T-Zellen/μl.

15.5 Richtigkeit

EDTA-Vollblutproben wurden mit dem CD4 easy count kit markiert und im CyFlow™ Counter analysiert. Die absoluten CD4-Werte wurden mit Ergebnissen eines BD FACSCalibur unter Verwendung von BD Tritest CD3/CD4/CD45 und BD Trucount-Röhrchen verglichen. Es zeigte sich eine Abweichung von -22,56 % zwischen den Methoden.

16 Grenzen des Verfahrens

VORSICHT ⚠ *Die Sysmex Partec CD4 easy count kits (05-8401) wurden nicht für die Verwendung bei Kindern und Jugendlichen validiert.*

VORSICHT ⚠ *Patientenmessungen von CD4 und CD4% mithilfe von Sysmex Partec Assays sollten nicht zusammen mit Methoden anderer Hersteller zur Bestimmung von CD4 und CD4% verwendet werden. Die Assays sollten nur mit den Sysmex Partec Geräten verwendet werden. Die Messwerte sind unter Umständen nicht beliebig austauschbar mit Werten, die mit anderen Geräten oder Assays gemessen wurden.*

Verschiedene endogene und exogene Substanzen wurden in Übereinstimmung mit der Richtlinie CLSI EP07-A2 auf eine mögliche Beeinträchtigung der Blutprobenanalyse untersucht.
Bei ≥ 18 % Hämolyse (gemäß dem in der Richtlinie CLSI EP07-A2, Anhang G empfohlenen Verfahren G1 „Osmotic Shock Procedure“ induziert) wurde eine Beeinträchtigung beobachtet. Aus diesem Grund sollten hämolytische Proben verworfen werden.
Bei weiteren Fragen hinsichtlich der Leistung des Produkts wenden Sie sich bitte an Ihre nächstgelegene Sysmex-Vertretung.

17 Literatur

1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for Determining Percentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Kontaktinformationen des Herstellers

Hersteller
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Germany
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Versionsnummer des Benutzerhandbuchs und Ausgabedatum

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Ausgegeben von: Sysmex Partec GmbH

Das Sicherheitsdatenblatt zu diesem Produkt ist unter www.sysmex-partec.com/services erhältlich.

sysmex

Guide d’utilisation

Identification du réactif IVD

Nom du produit :

CD4 easy count kit

REF 05-8401

Contenu :

Fiole de CD4 mAb PE — REF 05-8401-01
Flacon de no lyse buffer (tampon sans lyse) — REF 05-8401-02

1 Spécification

Spécificité	CD4 humain
Isotype	IgG1
Contenu	100 tests
Fluorochrome	PE
Excitation λ (nm)	532 / 488
Émission maximale (nm)	578

2 Usage prévu

IVD Pour la réalisation de diagnostics in vitro

Le CD4 easy count kit est un test IVD quantitatif manuel constitué de deux composants et destiné au marquage des sous-populations de lymphocytes dans le sang veineux total EDTA chez l’adulte, puis à leur dénombrement à l’aide du cytomètre en flux IVD CyFlow™ Counter de Sysmex Partec.
La concentration en cellules T CD4 des échantillons de sang est un indicateur utile pour l’initiation ou le suivi du traitement des porteurs du VIH, conjointement avec d’autres résultats cliniques et de laboratoire.
Le test doit être effectué par des professionnels de santé formés à cet effet.

3 Principe de la méthode d’examen

Une aliquote d'un échantillon de sang total d'EDTA est mélangée à l'anticorps (CD4) conjugué au fluorochrome dans un rapport 1:1, Après un temps d’incubation déterminé, le tampon est ajouté et l’échantillon est prêt pour l’analyse sur un cytomètre en flux CyFlow™ Counter.
La source lumineuse excite la teinture fluorescente liée à la cellule marquée et la lumière émise est détectée pendant le passage d’un certain volume d’échantillon de sang dans l’instrument. Le logiciel intégré calcule la concentration de la population cellulaire spécifique.
Pour de plus amples informations, se reporter au guide d’utilisation du CyFlow™ Counter (CY-S-3022IFUEN).

4 Stockage et durée de conservation après la première ouverture

1. Stockage :
Stocker l’anticorps et les réactifs tampons à une température comprise entre 2 et 8 °C à l’abri de la lumière. Ne pas congeler ni exposer les réactifs à des températures élevées et les protéger du rayonnement solaire direct.
2. Durée de conservation après la première ouverture :
Toujours fermer le flacon après usage et utiliser de nouveaux embouts de pipettes à chaque fois qu’un échantillon de réactif est prélevé pour éviter toute contamination.
Conserver l’anticorps et le réactif tampon à une température comprise entre 2 et 8 °C à l’abri de la lumière. Ne pas congeler ni exposer les réactifs à des températures élevées et les protéger du rayonnement solaire direct.
Dans ces conditions de stockage, le CD4 easy count kit restera stable jusqu’à la date de péremption imprimée sur l’étiquette du kit.

5 Composants

Le CD4 mAb PE est un anticorps monoclonal murin fourni dans un tampon PBS avec 0,2 % de BSA et 0,09 % d’azoture de sodium. Le tampon sans lyse est une solution à base de PBS contenant 0,09 % d’azoture de sodium.

6 Signes de détérioration

La solution d’anticorps et la solution tampon sont des liquides clairs. Ne pas utiliser les réactifs en cas d’apparition d’un quelconque signe de turbidité ou de contamination.
Pour toutes questions concernant la performance ou la qualité du produit reçu, veuillez contacter votre représentant Sysmex local.

7 Avertissements et mesures de précaution

Les réactifs contiennent de l’azoture de sodium 14 mM comme agent conservateur. La faible concentration de l‘azoture de sodium ne nécessite aucun marquage comme substance dangereuse, mais les mesures de sécurité normales relatives à la manipulation des produits chimiques doivent être observées. Se reporter à la fiche de données de sécurité pour obtenir la liste complète des avertissements et mesures de précaution.

8 Équipement supplémentaire requis

Instruments requis	CyFlow™ Counter (REF No.: CY-S-3022)
Matériel requis	Tubes d’échantillon (REF No.: 04-2000) Une pipette 20 μl validée et embouts de pipette Une pipette 100 – 1000 μl validée et embouts de pipette Système de prélèvement de sang veineux avec EDTA comme anticoagulant Chronomètre

9 Préparation des réactifs

CD4 mAb PE (05-8401-01), le réactif à base d’anticorps est prêt à l’emploi.
no lyse buffer (05-8401-02), la solution tampon est prête à l’emploi.

10 Prélèvement, manipulation et stockage de l’échantillon primaire

AVERTISSEMENT ⚠ *Tous les échantillons et matériaux biologiques sont considérés comme biologiquement dangereux et doivent être manipulés comme s’ils étaient susceptibles de transmettre une infection. Les mesures de sécurité et les procédures de manipulation appropriées doivent être appliquées conformément aux lois et aux réglementations en vigueur.*

- L’échantillon de sang doit être un échantillon de sang veineux total, prélevé à l’aide d’un système de prélèvement sanguin contenant de l’EDTA comme anticoagulant.
- L’échantillon de sang doit être transporté dans un récipient sombre, hermétique à la lumière, et ne doit pas être exposé à des températures élevées. Une boîte en polystyrène avec un dispositif de réfrigération, par exemple, constitue un conteneur de transport approprié. Les meilleures conditions de transport seraient inférieures à 25 °C si le transport ne prend pas plus de 6 heures après le don. Pour le transport et le stockage plus de 6 heures, le sang doit être conservé entre 2 et 8 °C.
- Ne pas congeler ni décongeler l’échantillon de sang.
- Pour des conditions optimales, l’échantillon de sang doit être frais, c’est-à-dire qu’il ne doit pas s’être écoulé plus de six heures entre le prélèvement de l’échantillon et l’analyse.
- Les échantillons de sang peuvent être utilisés jusqu’à 24 heures après la prise de sang, s’ils sont conservés au réfrigérateur à une température comprise entre 2 et 8 °C.
- Avant l’utilisation, les tubes d’échantillon de sang doivent être retournés doucement 8 à 10 fois.
- Ne pas utiliser des échantillons de sang coagulé.

11 Procédure d’examen

REMARQUE *Le pipettage à l’envers est essentiel à la précision, tout particulièrement en cas de délivrance d’échantillons visqueux et de volumes d’échantillons très réduits. Pour le pipettage de sang total, nous recommandons l’utilisation d’une pipette électronique calibrée qui est préprogrammée pour fonctionner en mode de pipettage à l’envers.*
En l’absence d’une pipette électronique, suivre les présentes consignes pour procéder à un pipettage manuel à l’envers :
- *Enfoncer le bouton d’actionnement jusqu’à la deuxième butée. Laisser le bouton remonter entièrement, l’excès d’échantillon est aspiré dans la pointe.*
- *Enfoncer le bouton d’actionnement jusqu’à la première butée pour expulser un volume précis de sang tout en conservant l’excès d’échantillon dans la pointe.*
- *Éliminer la pointe de pipette contenant l’excès d’échantillon de sang dans une boîte à déchets médicaux.*

Prière de noter qu’il n’est pas nécessaire de procéder au pipettage à l’envers pour les anticorps ou les solutions tampons.

11.1 Marquage

- Renverser doucement l’échantillon de sang dans le tube de prélèvement de sang EDTA 8 à 10 fois.
- Pipeter 20 μl d’échantillon de sang total EDTA dans le fond d’un tube d’échantillon, en évitant de laisser un filet de sang sur la paroi intérieure du tube avec l’embout de pipette. Jeter la pointe de la pipette.
- Ajouter 20 μl de CD4 mAb PE directement dans l’échantillon de sang et mélanger doucement en évitant de laisser un filet de sang sur la paroi intérieure du tube avec l’embout de pipette. Jeter la pointe de la pipette.
- Exemple à ce moment ne pas vortexer.
- Incuber le mélange pendant 15 minutes à 15-30 °C dans l'obscurité.
- Ajouter 800 μl de no lyse buffer (tampon sans lyse) et mélanger brièvement au vortex ou tapoter doucement le tube afin de mélanger l’échantillon. Jeter la pointe de la pipette.
- Mélanger brièvement au vortex ou tapoter doucement le tube avant d’effectuer l’analyse à l’aide du CyFlow™ Counter.

REMARQUE *Les échantillons doivent être analysés dans un délai de 2 heures après l’ajout de no lyse buffer (tampon sans lyse).*

11.2 Analyse de l’échantillon

Pour obtenir des informations sur le fonctionnement de l’instrument avant l’analyse des échantillons, se reporter au guide d’utilisation du CyFlow™ Counter (CY-S-3022IFUEN). Les procédures de démarrage et la procédure de contrôle qualité interne doivent avoir été effectuées avec succès avant l’analyse de l’échantillon.
- Choisissez et chargez la configuration pour la mesure CD4 à partir de la barre de menu du CyFlow™ Counter.
- Insérer le tube d’échantillon avec l’échantillon de sang préparé dans l’orifice d’échantillonnage.
- Démarrer la mesure.
- Après l’analyse de l’échantillon, l’instrument s’arrête et se nettoie automatiquement.
- Retirer et éliminer l’échantillon de sang conformément aux procédures locales de sécurité en laboratoire relatives aux risques biologiques.

Ne pas démarrer de seconde mesure avec le même tube d’échantillon. S‘il faut répéter ou effectuer deux fois la mesure, préparer un second tube d’échantillon conformément au point 11.1.

11.3 Acquisition et analyse des données

L’analyse des données à l’aide du CyFlow™ Counter n’est possible que si le contrôle qualité interne de l’instrument a été effectué avec succès.
Consulter le guide d’utilisation du CyFlow™ Counter (CY-S-3022IFUEN) pour effectuer une procédure de démarrage correcte et la maintenance de l’appareil, si nécessaire.
Le résultat du comptage des cellules T CD4 s’affiche à l’écran.
Si l’histogramme n’indique pas un pic clair des cellules T marquées positives CD4, il faut recommencer la préparation et la mesure de l’échantillon.
Les résultats seront incorrects si le positionnement de la fenêtre électronique (gate) n’est pas réalisé avec précision.
Prendre garde à ne pas fenêtrer les monocytes en tant que cellules marquées positives CD4, si la concentration en CD4 dans l’échantillon de sang du patient est extrêmement faible.

12 Calcul des résultats d’examen

Le calcul de la concentration cellulaire fait partie intégrante de l’analyse des données par logiciel qui fournit le résultat en cellules CD4 par μl d’échantillon de sang, après chaque mesure.
Pour de plus amples informations, se reporter au guide d’utilisation du CyFlow™ Counter (CY-S-3022IFUEN).

13 Interprétation des résultats

Le virus de l'immu-nodéficience hu-maine (VIH) est l'une des princi-pales raisons de la déplétion des cellules CD4, qui affai-blit le système im-munitaire. Les ré-sultats doivent être interprétés par les médecins, en liai-son avec les directives de traitement du VIH applicables localement.

14 Procédure de contrôle

Un contrôle qualité approprié doit être effectué conformément aux réglementations des laboratoires nationaux.
Il est recommandé d’utiliser un échantillon de sang de contrôle (05-8993 Control Blood-dry), avec une quantité spécifique de cellules CD4 pour le contrôle qualité quotidien de la préparation de l’échantillon et du comptage de l’instrument.

15 Caractéristiques de performance

15.1 Spécificité

L’anticorps monoclonal de souris MEM-241 reconnaît l’antigène CD4 humain, une glycoprotéine transmembranaire (55 kDa) de la superfamille des immunoglobulines présente sur une souspopulation de lymphocytes T (cellules T « auxiliaires/inductrices ») et exprimée à un niveau moindre sur les monocytes, les macrophages tissulaires et les granulocytes. HCDM (former HLDA VIII) Meeting, mai 2006, Québec, Canada ; WS Code M241

15.2 Capacité de détection

Les évaluations de la limite du blanc (LoB), de la limite de détection (LoD) et de la limite de quantification (LoQ) ont été réalisées conformément aux spécifications de la directive CLSI EP17-A2.
LoB : ≤ 50 % de la LoD
LoD : ≤ 20 cellules CD4/μl
LoQ : ≤ 40 cellules CD4/μl

15.3 Répétabilité

Variation intra- et inter-essai :
Les valeurs du coefficient de variation (CV) sont inférieures à 10 % pour des valeurs de CD4 supérieures ou égales à 200 cellules T CD4/μl.
Les valeurs du coefficient de variation (CV) sont inférieures à 15 % pour des valeurs absolues de CD4 inférieures à 200 cellules T CD4/μl.

15.4 Linéarité

La plage de mesure de l’essai et la linéarité de la mesure sont valides sur la plage :
taux absolu de CD4 : 40–2500 cellules T CD4/μl.

15.5 Justesse

Les échantillons de sang total EDTA ont été marqués avec le CD4 easy count kit et analysés sur le CyFlow™ Counter. Les valeurs absolues de CD4 ont été comparées aux résultats obtenus à l’aide d’un BD FACSCalibur en utilisant des réactifs BD Tritest CD3/CD4/CD45 et des tubes BD Trucount, ce qui a révélé un écart de -22,56 % entre les deux méthodes.

16 Limites de la procédure

PRUDENCE ⚠ *L’utilisation des kits CD4 easy count (05-8401) Sysmex Partec n’a pas été validée chez les patients pédiatriques et adolescents.*

PRUDENCE ⚠ *Les mesures du taux de CD4 et du pourcentage CD4% réalisées chez des patients à l’aide de tests Sysmex Partec ne doivent pas être utilisées de manière indépendante avec d’autres méthodes de fabricant visant à la détermination du taux de CD4 et du pourcentage CD4%. N’utiliser les tests qu’avec les instruments Sysmex Partec. Les valeurs obtenues à l’aide d’autres équipements ou tests sont susceptibles de ne pas être interchangeables.*

Différentes substances endogènes et exogènes ont été testées conformément à la directive CLSI EP07-A2 pour vérifier les éventuelles interférences pouvant avoir une influence sur l’analyse de l’échantillon de sang. Il a finalement été observé ≥ 18 % d’hémolyse (engendrée par l’utilisation du protocole recommandé dans la directive CLSI EP07-A2, annexe G, G1 – Procédure de choc osmotique) provoquant des interférences. C’est pourquoi les échantillons hémolysés devrait être écartés.
Pour toutes questions supplémentaires concernant la performance du produit, veuillez contacter votre représentant Sysmex local.

17 Bibliographie

1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
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18 Coordonnées du fabricant

Fabricant
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Germany
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Numéro de version du guide d’utilisation et date de publication

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La fiche de données de sécurité de ce produit est disponible à l’adresse suivante : www.sysmex-partec.com/services.

IVD CE

Instrucciones de uso

Identificación del reactivo para DIV

Nombre del producto:

Kit CD4 easy count

REF 05-8401

Contenido:

Vial con mAb CD4-PE REF 05-8401-01
Botella con solución amortiguadora sin lisis REF 05-8401-02

1 Descripción

Especificidad	CD4 humano
Isotipo	IgG1
Contenido	100 tests
Fluorocromo	PE
λ de excitación (nm)	532 / 488
Emisión máxima (nm)	578

2 Uso previsto

IVD Para diagnóstico in vitro.
El kit CD4 easy count es un test cuantitativo manual de DIV de dos componentes para el marcaje de subpoblaciones de linfocitos en sangre entera venosa adulta con EDTA y la subsiguiente enumeración con el citómetro de flujo para DIV CyFlow™ Counter de Sysmex Partec. La concentración de linfocitos T CD4 de las muestras de sangre es un indicador útil para iniciar o supervisar el tratamiento de personas infectadas por el VIH junto con otros hallazgos clínicos y de laboratorio.
El test está previsto para su uso por parte de profesionales sanitarios con la formación correspondiente.

3 Principio del método de análisis

Se mezcla una alícuota de una muestra de EDTA de sangre completa con el anticuerpo (CD4) conjugado con el fluorocromo en una proporción 1:1, Tras un tiempo fijo de incubación, se añade la solución amortiguadora y la muestra está lista para su análisis en un citómetro de flujo CyFlow™ Counter.
La fuente de luz excita el colorante fluorescente unido a la célula teñida; se detecta la luz emitida durante el paso de un determinado volumen de sangre a través del instrumento. La concentración de la población celular específica se calcula con el software integrado.
Para más información, consulte las instrucciones de uso de CyFlow™ Counter (CY-S-3022IFUEN).

4 Almacenamiento y tiempo de conservación tras abrir por primera vez

1. Almacenamiento:
Almacene los reactivos de anticuerpos y solución amortiguadora en un lugar oscuro a 2-8 °C. No congele ni exponga los reactivos a temperaturas elevadas o a la luz solar directa.
2. Tiempo de conservación tras abrir por primera vez:
Cierre siempre la botella después de su uso y utilice puntas de pipeta nuevas cada vez que tome muestras del reactivo para evitar la contaminación.
Mantenga el reactivo de anticuerpos y solución amortiguadora en un lugar oscuro a 2-8 °C. No congele ni exponga los reactivos a temperaturas elevadas o a la luz solar directa.
En estas condiciones de almacenamiento, el kit CD4 easy count permanecerá estable hasta la fecha de caducidad impresa en la etiqueta del kit.

5 Componentes

mAb CD4-PE es un anticuerpo monoclonal murino suministrado en una solución amortiguadora PBS con un 0,2% de SAB y un 0,09% de azida de sodio. La solución amortiguadora sin lisis es una solución con base de PBS que contiene un 0,09% de azida de sodio.

6 Indicios de deterioro

El reactivo de anticuerpos y la solución amortiguadora son líquidos transparentes. No utilice el reactivo si aparece cualquier tipo de turbidez o contaminación.
Si tiene alguna pregunta con respecto al funcionamiento o la calidad del producto recibido, contacte con su representante local de Sysmex.

7 Precaución y advertencias

Los reactivos contienen 14 mM de azida de sodio como conservante. Dada su reducida concentración de azida de sodio, no requiere un etiquetado como producto de riesgo, pero se deben observar las precauciones de seguridad normales para la manipulación de sustancias químicas. Consulte la ficha técnica de seguridad para obtener una lista completa de advertencias y precauciones.

8 Equipamiento adicional necesario

Instrumental necesario:	CyFlow™ Counter (REF No.: CY-S-3022)
Equipamiento requerido:	Tubos de ensayo de 3,5 ml (REF No.: 04-2000) Una pipeta verificada de 20 µl y puntas de pipeta Una pipeta verificada de 100-1000 µl y puntas de pipeta Sistema de extracción de sangre venosa con EDTA como anticoagulante Cronómetro

9 Preparación del reactivo

mAb CD4-PE (REF No.: 05-8401-01), el reactivo de anticuerpos está listo para su uso.
Solución amortiguadora sin lisis (REF No.: 05-8401-02), la solución amortiguadora está lista para su uso.

10 Principios básicos de extracción, manipulación y almacenamiento de muestras

Advertencia ⚠ *Todas las muestras y materiales biológicos se consideran material de riesgo biológico y deberían manipularse como si fueran potencialmente infecciosos. Se deben observar las precauciones de seguridad y procedimientos de manipulación adecuados conforme a la legislación y normativa.*

- La muestra de sangre debe ser una muestra de sangre entera venosa, extraída con un sistema de extracción de sangre que contenga EDTA como anticoagulante.
- La muestra de sangre debe transportarse en un contenedor oscuro, protegido de la luz y no expuesto a temperaturas elevadas. Un contenedor de transporte adecuado es, por ejemplo, una caja de poliestireno extruido con material refrigerante. Las mejores condiciones de transporte estarían por debajo de los 25 °C si el transporte no demorará más de 6 horas después de la donación. Para el transporte y el tiempo de almacenamiento de más de 6 horas, la sangre debe mantenerse a 2 - 8 °C.
- No se puede congelar y descongelar la muestra de sangre.
- En condiciones óptimas la muestra de sangre debería ser reciente, es decir, no deberían haber pasado más de seis horas entre la extracción y el análisis de la muestra.
- Las muestras de sangre se pueden utilizar hasta 24 horas después de la extracción si se almacenan en el frigorífico a 2-8 °C.
- Los tubos con las muestras de sangre se deben voltear con suavidad de 8 a 10 veces antes de ser utilizados.
- No utilice muestras de sangre coagulada.

11 Procedimiento de análisis

Aviso *El pipeteado inverso es fundamental para la precisión, en particular cuando se dispensan volúmenes de muestra viscosos y muy pequeños. Para el pipeteado de sangre completa, recomendamos emplear una pipeta electrónica calibrada que esté preprogramada para funcionar en modo de pipeteado inverso.*
Si no hay disponible una pipeta electrónica, siga las presentes instrucciones para llevar a cabo un pipeteado inverso manual:
- *Presione el botón de funcionamiento hasta el segundo tope. Deje que el botón de funcionamiento ascienda por completo; la muestra sobrante se aspirará a la punta.*
- *Presione el botón de funcionamiento hasta el primer tope para expulsar un volumen concreto de sangre, dejando así la muestra sobrante dentro de la punta.*
- *Elimine la punta de la pipeta que contiene la muestra de sangre sobrante en un contenedor para residuos médicos.*

Tenga en cuenta que no debe utilizar el pipeteado inverso para soluciones de anticuerpos ni soluciones tampón.

11.1 Tinción

- Voltee con suavidad de 8 a 10 veces el tubo de extracción de sangre con EDTA que contiene la muestra de sangre.
- Pase con la pipeta 20 µl de la muestra de sangre entera con EDTA al fondo de un tubo de muestra y evite manchar la pared interior del tubo con la sangre de la punta de la pipeta. Deseche la punta de la pipeta.
- Añada 20 µl de mAb CD4-PE directamente en la muestra de sangre y mézclelo suavemente, evitando manchar la pared interior del tubo con la sangre de la punta de la pipeta. Deseche la punta de la pipeta.
- Muestra en este momento no lo hacen vórtice.
- Incubar la mezcla durante 15 minutos a 15-30 °C en la oscuridad.
- Añada 800 µl de la solución amortiguadora sin lisis y agite brevemente con la agitadora vorticial, o bien golpee suavemente el tubo para mezclar la muestra. Deseche la punta de la pipeta.
- Agite brevemente mediante agitadora vorticial, o bien golpee suavemente el tubo antes del análisis con el CyFlow™ Counter.

Aviso *Las muestras se deben analizar en un periodo máximo de 2 horas tras la adición de la solución amortiguadora sin lisis.*

11.2 Análisis de la muestra

En las instrucciones de uso de CyFlow™ Counter (CY-S-3022IFUEN) puede consultar el modo del empleo del equipo antes de analizar las muestras. Los procedimientos de puesta en marcha y el proceso de control de calidad interno deben completarse con éxito antes de iniciar el análisis de la muestra.
- Elija y cargue la configuración para la medición de CD4 desde la barra de menú del CyFlow™ Counter.
- Inserte el tubo de muestras con la muestra de sangre preparada en el acoplamiento para muestras.
- Inicie la medición.
- Tras el análisis de la muestra, el dispositivo se detiene y se limpia automáticamente.
- Retire y deseche la muestra de sangre conforme a los procedimientos locales de seguridad en laboratorio frente a riesgos biológicos.

No inicie una segunda medición con el mismo tubo de muestras. Si se requiere una repetición de la medición, se debe preparar un segundo tubo de muestras conforme a 11.1.

11.3 Adquisición y análisis de datos

El análisis de datos con CyFlow™ Counter solo es posible si el control de calidad interno del instrumento ha resultado satisfactorio.
Consulte las instrucciones de uso del CyFlow™ Counter (CY-S-3022IFUEN) para realizar, si fuera necesario, el procedimiento de inicio y mantenimiento del dispositivo de forma adecuada. El resultado del recuento de las células T CD4 aparecerá en la pantalla. Si el histograma no muestra un pico claro de las células T con tinción positiva para CD4, se deben repetir la preparación de la muestra y la medición. Los resultados serán incorrectos si no se realiza una acotación precisa. Asegúrese de no acotar monocitos como células con tinción positiva para CD4 si la concentración de CD4 es extremadamente baja en la muestra de sangre de un paciente.

12 Cálculo de los resultados del análisis

El cálculo de la concentración celular forma parte del análisis de datos informático, el cual suministra el resultado en células CD4 por µl de muestra de sangre después de cada medición.
Para más información, consulte las instrucciones de uso de CyFlow™ Counter (CY-S-3022IFUEN).

13 Interpretación de los resultados

El virus de la inmu-nodeficiencia hu-mana (VIH) es uno de los principales motivos del ago-tamiento de las células CD4, que debilita el sistema inmunitario. Las respuestas deben ser interpretadas por los médicos, en conjunción con las pautas de trata-miento del VIH aplicables local-mente.

14 Procedimiento de control

Se debe realizar un control de calidad apropiado conforme a las normativas nacionales para laboratorios.
Se han comercializado diversas muestras de sangre estabilizada, pero solo unas pocas son adecuadas cuando se utilizan determinados citómetros de flujo.
Si no se cumple el criterio de aceptación del material de control específico, no prosiga con el análisis de la muestra del paciente y póngase en contacto con su representante local de Sysmex.
Para más información sobre el uso de material de control adecuado, póngase en contacto con su representante local de Sysmex.

15 Eficacia diagnóstica

15.1 Especificidad

El anticuerpo monoclonal murino MEM-241 reconoce el antígeno humano CD4, una glucoproteína transmembranaria (55 kDa) de la superfamilia de las inmunoglobulinas, presente en un subconjunto de linfocitos T (células T cooperadoras/inductoras) y expresada en menor medida en monocitos, macrófagos y granulocitos. Congreso de HCDM (anteriormente HLDA VIII), mayo de 2006, Quebec, Canadá. Código WS: M241.

15.2 Capacidad de detección

La evaluación del límite de inclusión (LI), el límite de detección (LD) y el límite de cuantificación (LC) se realizó de acuerdo con las indicaciones de la directriz CLSI EP17-A2.
LI: ≤50% del LD
LD: ≤20 células CD4/µl
LC: ≤40 células CD4/µl

15.3 Repetibilidad

Variación intra e interanalítica:
Los valores de volumen celular suponen menos del 10% para valores de CD4 ≥ 200 células T CD4/µl.
Los valores de volumen celular suponen menos del 15% para valores absolutos de CD4 inferiores a 200 células T CD4/µl.

15.4 Linealidad

El intervalo de medición del ensayo y la linealidad de la medición son válidos en el intervalo:
CD4 absoluto: 40–2500 células T CD4/µl.

15.5 Veracidad

Las muestras de sangre entera con EDTA se tiñeron con el kit CD4 easy count y se analizaron en el CyFlow™ Counter. Se compararon los valores de CD4 absoluto con los resultados de un BD FACSCalibur utilizando BD Tritest CD3/CD4/CD45 y tubos BD Trucount y se puso de manifiesto una divergencia del -22,56% entre ambos métodos.

16 Limitaciones

Cuidado ⚠ *El uso de los kits de Sysmex Partec CD4 easy count (05-8401) no está validado para pacientes pediátricos y adolescentes.*

Cuidado ⚠ *Las mediciones en pacientes de CD4 y CD4% mediante las pruebas de Sysmex Partec no deben usarse independientemente para determinar CD4 y CD4% con métodos de otros proveedores. Utilizar las pruebas exclusivamente con los instrumentos de Sysmex Partec. Es posible que los valores obtenidos mediante otros equipos o pruebas no sean intercambiables.*

Se analizaron diferentes sustancias endógenas y exógenas conforme a la directriz CLSI EP07-A2 para detectar posibles efectos de interferencia que pudieran influir en el análisis de la muestra de sangre. Finalmente, se observó un ≥18% de hemólisis (provocada por el uso del protocolo recomendado en CLSI EP07-A2, apéndice G, G1, «Procedimiento de choque osmótico») como muestra de un efecto de interferencia. Por ese motivo, se deben rechazar las muestras hemolizadas.
Si tiene alguna pregunta más con respecto al funcionamiento del producto, póngase en contacto con su representante local de Sysmex.

17 Referencias bibliográficas

1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
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4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for Determining Percentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
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18 Contacto del fabricante

Fabricante
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Alemanha
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Número de versión de las instrucciones de uso y fecha de publicación

Revisão: Rev-07_10-09-2020_CN 1563
Publicado por: Sysmex Partec GmbH

Puede consultar la ficha técnica de seguridad de este producto en www.sysmex-partec.com/services.

Instruções de utilização

Identificação do reagente IVD

Nome do produto:

CD4 easy count kit

REF 05-8401

Conteúdo:

Recipiente contendo CD4 mAb PE REF 05-8401-01
Frasco com tampão sem lise REF 05-8401-02

1 Especificacao

Especificidade	CD4 humano
Isotipo	IgG1
Conteudo	100 testes
Fluorocromo	PE
λ excitacao (nm)	532 / 488
Maximo de emissao (nm)	578

2 Uso previsto

IVD Para uso no diagnostico in vitro.
O CD4 easy count kit e um teste IVD manual quantitativo com dois componentes, para a marcacao de uma subpopulacao de linfocitos em sangue total EDTA venoso de adulto e enumeracao subsequente com o citometro de fluxo IVD Sysmex Partec CyFlow™ Counter. A concentracao de celulas T CD4 das amostras de sangue e um indicador util para a iniciacao ou o acompanhamento do tratamento de pacientes com VIH positivo em conjugacao com outros resultados laboratoriais e clinicos.
O teste destina-se a ser realizado por profissionais de saude devidamente qualificados.

3 Principio do metodo de analise

Uma alíquota de uma amostra de sangue total com EDTA é misturada com o anticorpo (CD4) conjugado com o fluorocromo numa proporção de 1:1, Apos um tempo de incubacao fixo, o tampao e adicionado e a amostra fica pronta para analise num citometro de fluxo CyFlow™ Counter.
A fonte de luz excita o corante fluorescente ligado com a celula corada e a luz emitida e detetada enquanto um determinado volume de amostra de sangue estiver a fluir atraves do instrumento.
A concentracao da populacao de celulas especificas e calculada pelo software integrado.
Para mais informacoes, consulte as instrucoes de utilizacao do CyFlow™ Counter (CY-S-3022IFUEN).

4 Armazenamento e prazo de validade apos a primeira abertura

1. Armazenamento:
Armazene os reagentes tampao e de anticorpos entre 2 e 8 °C no escuro. Nao congele nem exponha os reagentes a temperaturas elevadas e mantenha-os protegidos da luz solar direta.
2. Prazo de validade apos a primeira abertura:
Feche sempre o frasco apos a utilizacao e utilize pontas de pipeta novas de cada vez que o reagente e amostrado, para evitar a contaminacao.
Guarde os reagentes tampao e de anticorpos entre 2 e 8 °C no escuro. Nao congele nem exponha os reagentes a temperaturas elevadas e mantenha-os protegidos da luz solar direta.
Sob estas condicoes de armazenamento, o CD4 easy count kit ira permanecer estavel ate a data de validade impressa no rotulo do kit.

5 Componentes

O CD4 mAb PE e um anticorpo monoclonal murino fornecido em tampao PBS com 0,2% de BSA e 0,09% de azida de sodio. O tampao sem lise e uma solucao baseada em PBS contendo 0,09% de azida de sodio.

6 Indicio de deterioracao

As solucoes tampao e de anticorpos sao liquidos transparentes. Nao utilize os reagentes caso surja qualquer tipo de turbidez ou contaminacao.
Para questoes relativas ao desempenho ou qualidade do produto recebido, entre em contacto com o representante local da Sysmex para obter suporte tecnico.

7 Precaucoes e advertencias

Os reagentes contem 14 mM de azida de sodio como conservante. A baixa concentracao de azida de sódio nao exige a rotulagem como substancia perigosa, mas e necessario respeitar as precaucoes de seguranca normais relativas ao manuseamento dos produtos quimicos. Consulte a ficha de seguranca para obter uma lista completa das advertencias e precaucoes.

8 Equipamento adicional necessario

Instrumento necessario:	CyFlow™ Counter (REF No.: CY-S-3022)
Equipamento necessario:	Tubos de amostra de 3,5 ml (REF No.: 04-2000) Uma pipeta verificada de 20 µl e pontas de pipeta Uma pipeta verificada de 100-1000 µl e pontas de pipeta Sistema de colheita de sangue venoso com EDTA como anticoagulante Cronometro

9 Preparacao do reagente

CD4 mAb PE (REF No.: 05-8401-01), o reagente de anticorpos esta pronto a usar.
Tampao sem lise (REF No.: 05-8401-02), a solucao tampao esta pronta a usar.

10 Colheita, manuseamento e armazenamento da amostra principal

Advertencia ⚠ *Todas as amostras biologicas e materiais sao considerados como perigosos e devem ser manuseados como sendo capazes de transmitir infecoes. E necessario aplicar precaucoes de seguranca e procedimentos de manuseamento adequados em conformidade com as leis e regulamentos aplicaveis.*

- A amostra de sangue tem de ser uma amostra de sangue total venoso, recolhida com um sistema de colheita de sangue, que contem EDTA como anticoagulante.
- A amostra de sangue tem de ser transportada num recipiente escuro, protegida da luz e nao exposta a temperaturas elevadas. Um recipiente de transporte adequado e, p. ex., uma caixa de espuma de poliestireno com material de refrigeracao. As melhores condições de transporte seriam inferiores a 25 °C se o transporte não durar mais de 6 horas após a doação. Para transporte e tempo de armazenamento superior a 6 horas, o sangue deve ser mantido a 2 - 8 °C.
- Nao congele e descongele a amostra de sangue.
- A fim de assegurar as melhores condicoes, a amostra de sangue deve ser fresca, ou seja, o tempo de colheita e analise da mesma nao pode ser superior a seis horas.
- As amostras de sangue podem ser utilizadas ate 24 horas apos a colheita, desde que tenham sido armazenadas no frigorifico entre 2 e 8 °C.
- Antes da utilizacao, os tubos de amostra que contem o sangue devem ser invertidos cuidadosamen e 8 a 10 vezes.
- Nao utilize amostras de sangue coagulado.

11 Procedimento de analise

Nota *A pipetagem reversa é fundamental para a precisão, sobretudo ao dispensar volumes de amostra viscosos e muito reduzidos. Para a pipetagem de sangue total, recomendamos a utilização de uma pipeta eletrónica calibrada pré-programada para operar em modo de pipetagem reversa.*
Se não estiver disponível uma pipeta eletrónica, seguir estas instruções para a pipetagem reversa manual:
- *Premir o botão de operação até ao segundo ponto de retenção. Deixar o botão de operação subir completamente; o excesso da amostra é aspirado para a ponta.*
- *Premir o botão de operação até ao primeiro ponto de retenção para expulsar um volume exato de sangue, deixando o excesso da amostra na ponta.*
- *Deitar a ponta da pipeta com a amostra de sangue em excesso no contentor de lixo hospitalar.*

Ter em atenção que a pipetagem reversa não pode ser usada para anticorpos ou soluções-tampão.

11.1 Coloracao

- Inverta cuidadosamente a amostra de sangue no tubo de colheita de sangue EDTA 8 a 10 vezes.
- Pipete 20 µl de amostra de sangue total EDTA ate ao fundo de um tubo de amostra, evitando deixar um rasto de sangue na parede interior do tubo com a ponta de pipeta. Descarte a ponta da pipeta.
- Adicione 20 µl de CD4 mAb PE diretamente na amostra de sangue e misture cuidadosamente, evitando deixar um rasto de sangue na parede interior do tubo com a ponta de pipeta. Descarte a ponta da pipeta.
- Amostra neste momento nao vortice.
- Incubar a mistura por 15 minutos a 15-30 °C no escuro.
- Adicione 800 µl de tampao sem lise e centrifugue brevemente ou bata cuidadosamente no tubo para misturar a amostra. Descarte a ponta da pipeta.
- Centrifugue brevemente ou bata cuidadosamente no tubo antes de efetuar a analise com o CyFlow™ Counter.

Nota Apos a adicao do tampao sem lise, as amostras tem de ser analisadas dentro de 2 horas.

11.2 Analise da amostra

Consulte as instrucoes de utilizacao do CyFlow™ Counter para obter informacoes sobre como operar o instrumento antes de analisar as amostras. Os procedimentos de arranque e o procedimento interno de controlo da qualidade tem de ser concluidos com exito antes da analise da amostra.
- Escolha e carregue a configuração para medição de CD4 na barra de menu do CyFlow™ Counter.
- Insira o tubo da amostra com a amostra de sangue preparada na porta de amostragem.
- Inicie a medicao.
- Apos o processamento da amostra, o instrumento para e limpa-se automaticamente.
- Retire e elimine a amostra de sangue de acordo com os procedimentos de seguranca locais do laboratorio em termos de perigo biologico.

Nao inicie uma segunda medicao com o mesmo tubo de amostra. Se for necessaria uma duplicacao ou repeticao da medicao, tera de ser preparado um segundo tubo de amostra tal como descrito em 11.1.

11.3 Aquisicao e analise de dados

A analise de dados com o CyFlow™ Counter so e possivel caso o controlo de qualidade interno do instrumento tenha sido executado com exito.
Consulte as instrucoes de utilizacao do CyFlow™ Counter (CY-S-3022IFUEN) para informacoes sobre o procedimento de arranque e a manutencao devidos do dispositivo, se necessario.
O resultado da contagem de celulas T CD4 sera apresentado no visor.
Se o histograma nao apresentar um pico claro de celulas T coradas positivas CD4, a preparacao e medicao da amostra tem de ser repetidas.
Caso o posicionamento da placa nao seja efetuado com precisao, os resultados serao incorretos.
Caso a concentracao de CD4 seja extremamente baixa numa amostra de sangue do paciente, tenha o cuidado de nao colocar monocitos na placa em vez de celulas coradas positivas CD4.

12 Calculo dos resultados da analise

O calculo da concentracao de celulas faz parte da analise de dados baseada em software, que fornece o resultado em celulas CD4 por amostra de sangue µl, apos cada medicao.
Para mais informacoes, consulte as instrucoes de utilizacao do CyFlow™ Counter (CY-S-3022IFUEN).

13 Interpretacao dos resultados

O vírus da imunodeficiência humana (HIV) é uma das principais razões para a depleção de células CD4, que debilita o sistema imune. Os resultados devem ser interpretados pelos médicos, em conjunção com as diretrizes locais de tratamento do HIV.

14 Procedimento de controlo

Deve ser realizado um controlo da qualidade apropriado de acordo com regulamentos laboratoriais nacionais.
Encontra-se disponivel no mercado uma variedade de amostras de sangue estabilizado, mas poucas sao adequadas para a utilizacao de certos citometros de fluxo.
Se nao for cumprido o criterio de aceitacao do material de controlo individual, nao avance para o teste da amostra do paciente e entre em contacto com o seu representante local da Sysmex.
Para mais informacoes acerca do uso de material de controlo adequado, entre em contacto com o seu representante local da Sysmex.

15 Caracteristicas de desempenho

15.1 Especificidade

O anticorpo monoclonal do rato MEM-241 reconhece o antigenio CD4 humano, uma glicoproteina transmembrana (55 kDa) da familia do supergene da imunoglobulina, presente num subconjunto de linfocitos T (celulas T .auxiliares/indutorasg) e expressa a um nivel inferior em monocitos, granulocitos e macrofagos do tecido. Conferencia sobre HCDM (o anterior HLDA VIII), maio de 2006, Quebeque, Canada; codigo WS M241

15.2 Capacidade de detecao

A avaliacao para Limite de valores em branco (LoB), Limite de detecao (LoD) e Limite de quantificacao (LoQ) foi realizada de acordo com as especificacoes da diretriz CLSI EP17-A2.
LoB: ≤50% de LoD
LoD: ≤20 celulas CD4/µl
LoQ: ≤40 celulas CD4/µl

15.3 Repetibilidade

Variacao intra e entre ensaios:
Os valores CV sao inferiores a 10% para valores CD4 de 200 celulas T CD4/µl ou superiores.
Os valores CV sao inferiores a 15% para valores absolutos de CD4 inferiores a 200 celulas T CD4/µl.

15.4 Linearidade

A gama de medicao do ensaio e linearidade da medicao sao validas ao longo da gama:
CD4 absoluto: 40-2500 celulas T CD4/µl

15.5 Veracidade

Uma amostra de sangue total EDTA foi corada com o CD4 easy count kit e analisada no CyFlow™ Counter. Os valores de CD4 absoluto foram comparados com os resultados de um BD FACSCalibur usando BD Tritest CD3/CD4/CD45 e tubos BD Trucount, tendo-se revelado um desvio de -22,56% entre ambos os metodos.

16 Limitacoes

Cuidado ⚠ *Os kits Sysmex Partec CD4 easy count (05-8401) nao foram validados para utilizacao com pacientes pediatricos e adolescentes.*

Cuidado ⚠ *Os medicoes de paciente de CD4 e de CD4% feitas com os ensaios Sysmex Partec nao devem ser usadas de forma independente com outros metodos do fabricante para determinar CD4 e CD4%. Use os ensaios apenas com os instrumentos Sysmex Partec. Os valores obtidos usando outros equipamentos ou ensaios podem nao ser intercambiaveis.*

A Foram testadas diversas substancias endogenas e exogenas relativamente a diretriz CLSI EP07-A2 para identificar possiveis efeitos de interferencia que possam exercer influencia sobre a analise da amostra de sangue.
Finalmente, observouse . 18% de hemolise (induzida usando o protocolo recomendado na CLSI EP07-A2, Apendice G, G1 - Procedimento de Choque Osmotico) para demonstrar um efeito de interferencia. Por esse motivo, deverao ser rejeitadas amostras hemolisadas. Para outras questoes relativas ao desempenho do produto, entre em contacto com o seu representante local da Sysmex.

17 Referencias bibliograficas

1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondiere, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter®could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
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18 Contacto do fabricante

Fabricante
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Alemanha
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Numero de versao das instrucoes de utilizacao e data da pu blicacao

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Publicado por: Sysmex Partec GmbH

A ficha de seguranca deste produto esta disponivel em www.sysmex-partec.com/services.

Инструкция по эксплуатации

Идентификация реагента для диагностики in vitro (IVD)

Наименование продукта

CD4 easy count kit

REF 05-8401

Содержимое

Ампула, содержащая CD4 mAb PE REF 05-8401-01
Флакон, содержащий нелизирующий буфер REF 05-8401-02

1 Спецификация

Специфичность	CD4 человека
Изотип	IgG1
Содержимое	100 тестов
Флуорохром	PE
λ возбуждение (нм)	532 / 488
Максимальная эмиссия (нм)	578

2 Предполагаемое использование

IVD Для использования в диагностике in vitro.
Набор CD4 easy count kit – это двухкомпонентный набор, предназначенный для ручного количественного анализа in vitro, используемый для мечения субпопуляций лимфоцитов в цельной венозной крови с ЭДТА взрослых пациентов и их последующего подсчета с помощью проточного цитометра для диагностики in vitro CyFlow™ Counter компании Sysmex Partec.
Концентрация CD4 T-клеток в образцах крови является полезным показателем для начала или продолжения лечения ВИЧ-положительных пациентов наряду с другими лабораторными и клиническими данными.
Анализ должен проводиться обученными специалистами здравоохранения.

3 Принцип метода подсчета

Аликвоту образца цельной крови EDTA смешивают с антителом (CD4), конъюгированным с флуорохромом, в соотношении 1:1, После инкубирования в течение фиксированного времени добавляют буферный раствор, после чего образец готов для анализа на проточном цитометре CyFlow™ Counter.
Источник света активирует флуоресцентный краситель, которым мечена клетка, и испускаемый красителем свет определяется во время анализа образца крови определенного объема в приборе. Концентрация клеток определенной популяции рассчитывается с помощью интегрированного программного обеспечения. Для получения дополнительной информации см. ИЭ цитометра CyFlow™ Counter (CY-S-3022IFUEN).

4 Хранение и срок годности после первого вскрытия

1. Хранение.
Антитела и буферные реагенты следует хранить при температуре 2 – 8°C в темном месте. Реагенты нельзя замораживать, подвергать воздействию высоких температур и прямых солнечных лучей.
2. Срок годности после первого вскрытия.
Следует всегда закрывать флакон после использования и каждый раз при взятии реагента пользоваться новым наконечником для пипетки с целью предотвращения контаминации.
Антитела и буферные реагенты следует хранить при температуре 2 – 8°C в темном месте. Реагенты нельзя замораживать, подвергать воздействию высоких температур и прямых солнечных лучей.
При таких условиях хранения реагенты набора CD4 easy count kit будут стабильны до окончания срока годности, указанного на его этикетке.

5 Компоненты

CD4 mAb PE — это мышиные моноклональные антитела в фосфатно-солевом буфере (PBS), содержащем 0,2 % БСА и 0,09 % азида натрия. Нелизирующий буфер представляет собой раствор на основе PBS, содержащий 0,09 % азида натрия.

6 Признаки ухудшения качества

Антитела и буферные растворы представляют собой прозрачную жидкость. Не следует использовать реагенты при появлении любого рода помутнения или признаков контаминации. При наличии вопросов о характеристиках или качестве продукта обращайтесь в службу технической поддержки местного представительства компании Sysmex.

7 Предупреждения и меры предосторожности

Реагенты содержат 14 мМ азида натрия в качестве консерванта. Низкая концентрация азида натрия не требует маркировки опасностей, однако при их использовании следует соблюдать стандартные меры предосторожности по безопасному обращению с химическими веществами. Полный перечень предупреждений и мер предосторожности приведен в паспорте безопасности (ПБ) продукта.

8 Дополнительное требуемое оборудование

Требуемый прибор:	CyFlow™ Counter (REF No.: CY-S-3022)
Требуемое оборудование:	пробирки объемом 3,5 мл (REF No.: 04-2000) поверенная пипетка объемом 20 мкл и наконечники для пипетки поверенная пипетка объемом 100–1000 мкл и наконечники для пипетки система для сбора венозной крови с ЭДТА в качестве антикоагулянта секундомер

9 Приготовление реагента

CD4 mAb PE (REF No.: 05-8401-01), Реагент с антителами готов к использованию.
нелизирующий буфер (REF No.: 05-8401-02), Буферный раствор готов к использованию.

10 Взятие основного образца, обращение с ним и его хранение

ПРЕДУПРЕЖДЕНИЕ ⚠ *Все биологические образцы и материалы считаются биологически опасными, с ними следует обращаться как с потенциальным источником инфекции. При обращении с ними следует соблюдать надлежащие меры предосто-рожности и требования безопасности в соответствии с применимыми законами и нормативами.*

- Образцом должен служить образец цельной венозной крови, взятый с помощью системы для сбора крови, содержащей ЭДТА в качестве антикоагулянта.
- Образец крови следует транспортировать в непрозрачном контейнере, защищать от воздействия света и не подвергать воздействию высоких температур. Подходящим транспортным контейнером является, например, пенопластовый (Styrofoam) контейнер с охлаждающим материалом. Лучшие транспортные условия будут ниже 25 °C, если транспорт не займет больше 6 часов после пожертвования. Для транспортировки и хранения более 6 часов кровь должна храниться при температуре 2-8 °C.
- Нельзя замораживать и размораживать образец крови.
- Для получения наилучших результатов образец крови должен быть свежим, т. е. между его взятием и анализом должно пройти не более шести часов.
- Образцы крови можно использовать в течение 24 часов после взятия, если они хранятся в холодильнике при температуре 2 – 8°C.
- Перед началом анализа пробирку с образцом крови следует аккуратно перевернуть от 8 до 10 раз.
- Запрещается использование свернувшихся образцов крови.

11 Процедура анализа

ПРИМЕЧАНИЕ *Обратное пипетирование крайне важно для обеспечения точности, особенно при дозировании проб очень малого объёма. Рекомендуется использовать калиброванную электронную пипетку, запрограммированную на работу в режиме обратного пипетирования.*
Если электронная пипетка недоступна, выполните обратное пипетирование вручную следующим образом:
- *нажмите на кнопку управления до второго упора. Дайте кнопке управления полностью подняться, при этом избыток пробы втягивается в кончик.*
- *нажмите на кнопку управления до первого упора, чтобы выдавить точный объём крови, оставив избыток пробы в кончике.*
- *Выбросьте кончик пипетки с избытком пробы крови в контейнер для медицинских отходов.*

Обратите внимание, что обратное пипетирование нельзя использовать для растворов антител и буферных растворов.

11.1 Окрашивание

- Аккуратно переверните пробирку с ЭДТА и образцом крови от 8 до 10 раз.
- Перенесите пипеткой 20 мкл образца цельной крови с ЭДТА на дно пробирки для анализа, не оставляя наконечником пипетки следов крови на внутренней стенке пробирки. Отбросьте наконечник пипетки.
- Добавьте 20 мкл раствора антитела CD4 mAb PE непосредственно в образец крови и аккуратно перемешайте, не оставляя наконечником пипетки следов крови на внутренней стенке пробирки. Отбросьте наконечник пипетки.
- Образец в это время не завихрения.
- Инкубируйте смесь в течение 15 минут при температуре 15-30 °C в темноте.
- Добавьте 800 мкл нелизирующего буфера и перемешайте (недолго) содержимое пробирки вихревым способом или аккуратно постучите по пробирке. Отбросьте наконечник пипетки.
- Перед анализом в цитометре CyFlow™ Counter перемешайте содержимое пробирки вихревым способом или аккуратно постучите по пробирке.

ПРИМЕЧАНИЕ *После добавления нелизирующего буфера образцы должны быть проанализированы в течение 2 часов.*

11.2 Анализ образца

Для получения информации о работе с цитометром CyFlow™ Counter (CY-S-3022IFUEN) перед анализом образцов см. его ИЭ. Перед анализом образца следует успешно завершить предпусковые процедуры и процедуру внутреннего контроля качества.
- Выберите и загрузите конфигурацию для измерения CD4 из строки меню CyFlow™ Counter.
- Вставьте пробирку с приготовленным образцом крови в соответствующий держатель.
- Начните анализ.
- После анализа образца прибор прекращает работу и очищается автоматически.
- Извлеките и утилизируйте образец крови в соответствии с местными правилами по биологической безопасности при работе в лабораториях.

Не проводите повторный анализ с использованием той же самой пробирки с образцом. Если нужно выполнить дублирующее или повторное измерение, вторую пробирку для анализа подготовьте согласно п. 11.1.

11.3 Сбор и анализ данных

Анализ данных с помощью цитометра CyFlow™ Counter возможен только при успешном выполнении процедуры внутреннего контроля качества прибора.
Информация, необходимая для правильного запуска и технического обслуживания прибора, содержится в ИЭ цитометра CyFlow™ Counter (CY-S-3022IFUEN).
Результат определения количества CD4-T-клеток будет представлен на экране. Если на гистограмме отсутствует четкий пик положительно окрашенных CD4-T-клеток, следует приготовить новый испытуемый образец и повторить анализ.
Результаты будут неверными, если позиционирование области клеток будет выполнено не точно. Следует с осторожностью интерпретировать результаты анализа и не относить моноциты к положительно окрашенным CD4-клеткам, если концентрация CD4-клеток в образце крови пациента является очень низкой.

12 Расчет результатов анализа

Расчет концентрации клеток выполняется в рамках анализа данных программным обеспечением, что дает результат определения количества CD4-клеток на 1 мкл образца крови после каждого анализа. Для получения дополнительной информации см. ИЭ цитометра CyFlow™ Counter (CY-S-3022IFUEN).

13 Интерпретация результатов

Вирус иммунодефицита человека (ВИЧ) является одной из основных причин истощения клеток CD4, которая дебиллирует систему im-mune. Рецензии следует интерпретировать врачами в соответствии с местными рекомендациями по лечению ВИЧ.

14 Контрольная процедура

Следует проводить надлежащий контроль качества согласно национальным нормативным требованиям к проведению лабораторных исследований.
В продаже присутствуют различные стабилизированные образцы крови, однако только некоторые из них подходят для конкретных типов проточных цитометров.
В случае несоблюдения критерия приемлемости для отдельного контрольного материала не выполняйте исследование образца пациента и обратитесь к местному представителю компании Sysmex.
За дополнительной информацией об использовании подходящего контрольного материала обратитесь к местному представителю компании Sysmex.

15 Рабочие характеристики

15.1 Специфичность

Мышиное моноклональное антитело MEM-241 распознает антиген CD4 человека, транс мембранный гликопротеин (55 кДа), входящий в семейство супергена иммуноглобулинов, который экспрессируется на поверхности клеток одной из субпопуляций Т-лимфоцитов (Т-хелперы/Т-индукторы), а также, в меньших количествах, на поверхности моноцитов, тканевых макрофагов и гранулоцитов. Рабочее совещание по дифференцировочным молекулам клеток человека — HCDM (ранее VIII семинар по дифференцировочным антигенам лейкоцитов человека — HLDA), май 2006 г., Квебек, Канада; код WS M241

15.2 Способность обнаружения

Выполнялась оценка для предела холостой пробы (LoB), предела обнаружения (LoD) и предела количественного определения (LoQ) согласно спецификациям, указанным в руководстве CLSI EP17-A2.
LoB: ≤50% от LoD
LoD: ≤20 CD4-клеток/мкл
LoQ: ≤40 CD4-клеток/мкл

15.3 Воспроизводимость

Вариабельность в пределах одного и нескольких анализов:
Значения коэффициента вариации (CV) составляют менее 10 % для количества CD4-клеток, равного или превышающего 200 CD4-T-клеток на 1 мкл.
Значения коэффициента вариации (CV) составляют менее 15 % для абсолютного количества CD4-клеток ниже 200 CD4-T-клеток на 1 мкл.

15.4 Линейность

Диапазон измерений анализа и линейность анализа валидны в пределах следующего диапазона: абсолютное количество CD4-клеток: 40–2500 CD4-T-клеток на 1 мкл;

15.5 Правильность

Образцы цельной крови с ЭДТА окрашивали с помощью набора CD4 easy count kit и анализировали на цитометре CyFlow™ Counter. Абсолютное количество CD4-клеток сравнивали с результатами анализа на цитометре BD FACSCalibur с использованием реагентов BD Tritest CD3/CD4/CD45 и пробирок BD Trucount. Систематическое расхождение между методами составило -22,56 %.

16 Ограничения

ВНИМАНИЕ ⚠ *Наборы Sysmex Partec CD4 easy count (05-8401) не были валидированы для использования у пациентов детского и подросткового возраста.*

ВНИМАНИЕ ⚠ *Измерения CD4 и CD4%, проведённые при помощи анализов Sysmex Partec, не следует использовать независимо с методами определения CD4 и CD4% от других производителей. Используйте анализы только с приборами Sysmex Partec. Значения, полученные с использованием других приборов или анализов, могут быть неравнозначными.*

Различные эндогенные и экзогенные вещества были проверены в соответствии с руководством CLSI EP07-A2 на возможность искажения результатов анализа образца крови. В ≥ 18 % случаев гемолиза (вызванного применением протокола, рекомендованного в CLSI EP07-A2, приложение G, G1: метод осмотического шока) наблюдалось искажение результатов. По этой причине не следует использовать гемолизированные образцы. При наличии дополнительных вопросов о характеристиках продукта обратитесь к местному представителю компании Sysmex.

17 Литература

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18 Контактная информация производителя

Производитель
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Германия
www.sysmex-partec.com
Тел+49 3581 8746 0
Факс+49 3581 8746 70
Эл. почта:info@sysmex-partec.com

19 Номер редакции и дата выпуска ИЭ

Редакция: ред -07_10-09-2020_CN 1563
Выпущено: Sysmex Partec GmbH

Паспорт безопасности данного продукта доступен на веб-сайте www.sysmex-partec.com/services.

1.2 CD4 easy count kit (product code 05-8410)

IVD CE 0197

REF 05-8410

CD4 easy count kit

EN

Read and follow instructions carefully.
Note: Changes to previous version highlighted

1 Identification of the IVD reagent

Name	CD4 easy count kit	
Ref. No.	05-8410	
UDI-DI	04250878904832	
Content	Vial containing CD4 mAb PE	05-8410-01
	Bottle containing *no lyse buffer*	05-8410-02
	All components are ready to use.	

2 Specification

Specificity	Human CD4
Isotype	IgG1
Clone	Mouse MEM-241
Content	100 tests
Fluorochrome	PE
λ excitation (nm)	496 / 566
Emission maximum (nm)	576

3 Intended purpose

IVD For In Vitro Diagnostic Use.

The CD4 easy count kit is a two-component, quantitative IVD test for subpopulation labelling of lymphocytes in adult venous EDTA whole blood, and subsequent enumeration with a suitable Sysmex Partec IVD flow cytometer after manual sample preparation. The CD4 T cell concentration is useful to assess the immune and clinical status of patients. It is an indicator for the initiation or follow-up of treatment for people living with HIV, in conjunction with other laboratory and clinical findings. The test is intended to be performed by trained healthcare professionals.

4 Principle of the procedure

An aliquot of an EDTA whole blood sample is mixed with the antibody (CD4) conjugated to the fluorochrome in a 1:1 ratio. After a fixed incubation time, the buffer is added, and the sample is ready for analysis e.g. on the CyFlow™ Counter flow cytometer. The light source excites the fluorescent dye linked with the stained cell and the emitted light is detected while a precise volume of blood sample is running through the instrument. The concentration of the dedicated cell populations is calculated by the integrated software.

For further information, please refer to the instructions for use (IFU) of the suitable Sysmex Partec IVD flow cytometer.

5 Storage and shelf life

5.1 Unopened product

Store the antibody and buffer reagents at 2 - 8°C in the dark. Do not freeze or expose the reagents to elevated temperatures and keep them away from direct sunlight. Under these conditions the reagent kit will be stable until the expiration date printed on the label. Do not use the reagents after the expiration date.

5.2 Product after first opening

Always close the bottle after use and use new pipette tips each time the reagent is sampled to avoid contamination. The shelf life after first opening is the same as the shelf life for unopened reagents if stored at stated storage conditions and used according to the instructions above.

6 Components

CD4 mAb PE is a murine monoclonal antibody supplied in PBS buffer with 0.2% BSA and 0.09% sodium azide. *No lyse buffer* is a PBS-based solution containing 0.09% sodium azide.

7 Evidence of deterioration

The antibody and buffer solutions are clear liquids. Do not use the reagents after appearance of any kind of turbidity or contamination.

For questions regarding the performance or quality of the product, please contact your local Sysmex representative.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user is located.

8 Precautions and warnings

Reagents contain 0.09% sodium azide as a preservative. The low concentration of sodium azide does not require hazard labelling, but the normal safety precautions for the handling of chemicals must be observed. Refer to the Safety Data Sheet (SDS) for a complete list of warnings and precautions. Safety Data Sheet for this product is available at http://www.sysmex-partec.com/services, or at https://us.sysmex-flowcytometry.com/.

9 Additional required equipment

Instrument:	Suitable Sysmex Partec IVD flow cytometer, i.e., CyFlow™ Counter (Ref. No. CY-S-3022 or CY-S-3023)
Laboratory equipment:	Venous blood collection system with EDTA (K2 or K3) as anticoagulant
	Calibrated pipettes with disposable pipette tips for 20 and 100-1000 µL
	Sample tube(s) compatible with the flow cytometer
	Adequate personal protective equipment
	Stopwatch

Further materials may be required. Refer to the appropriate flow cytometer IFU for more information.

10 Disposal

Dispose of product after the expiration date in accordance with local regulations.

11 Primary sample collection, handling and storage

WARNING *All biological specimens and materials are considered biohazards and should be handled as if capable of transmitting infection. Appropriate safety precautions and handling procedures must be applied in accordance with applicable laws and regulations.*

- The blood sample must be a venous, whole-blood sample from a human adult, collected with a blood collection system which contains EDTA (K2 or K3) as anticoagulant.
- The blood sample must be transported in a container, protected from light and not exposed to elevated temperatures (e.g. a polystyrene box with cooling material).
- For best conditions, use fresh blood samples.
- Blood samples can be used up to 24 hours after drawing if they are stored at 2 - 8°C. Blood samples that have been transported and stored at up to 30°C must be analysed within 6 hours after drawing.
- Do not freeze and thaw the blood sample.
- Before use, gently invert the sample tubes containing the blood 8 to 10 times.
- Do not use clotted blood samples.

12 Examination procedure

NOTE *Reverse pipetting is critical to accuracy, especially when dispensing viscous and very small sample volumes. For whole blood pipetting, we recommend using a calibrated electronic pipette which is preprogrammed to operate in the reverse pipetting mode. If an electronic pipette is not available, follow these instructions for manual reverse pipetting:*

- *Depress the operating button to the second stop and place it in the homogenised specimen. Let the operating button move up completely. Excess sample is drawn up into the tip.*
- *Depress the operating button to the first stop to expel a precise volume of blood, leaving excess sample in the tip.*
- *Discard pipette tip with excess blood sample in the tip into medical waste container.*

Do not use reverse pipetting for antibody or buffer solution.

12.1 Quality control procedure

The CD4 easy count kit can be used without a biological control material. For information on the quality control procedure for the Sysmex Partec IVD flow cytometer, please refer to the corresponding instructions for use.

12.2 Staining

1. Invert the blood sample in the EDTA blood collection tube gently 8 to 10 times.
2. Pipette 20 µL EDTA whole blood sample to the bottom of an unused sample tube. Avoid leaving a trail of blood from the pipette tip on the inner tube wall. Discard the pipette tip.
3. Add 20 µL CD4 mAb PE directly into the blood sample and mix it gently. Avoid leaving a trail of blood from the pipette tip on the inner tube wall. Discard the pipette tip.
4. Incubate the mixture for a minimum of 15 minutes and a maximum of 30 minutes at 15 - 30°C in the dark.
5. Add 800 µL *no lyse buffer* and vortex briefly or mix the sample by pipetting up and down. Discard the pipette tip.
6. Proceed with sample analysis immediately.

NOTE *After addition of no lyse buffer samples can be stored for up to 2 hours at 2 - 8°C in the dark. Briefly vortex or mix the sample again by pipetting up and down immediately before analysis. Do not use samples that have exceeded maximum storage time.*

12.3 Sample analysis

Please refer to the IFU of the Sysmex Partec IVD flow cytometer for how to operate the instrument. The start-up procedures and the internal quality control procedure must be successfully completed before sample analysis. The following steps describe the sample analysis on a suitable CyFlow™ Counter.

1. Choose and load the configuration for CD4 measurement from the menu bar of the CyFlow™ Counter.
2. Insert the sample tube with the prepared blood sample into the sample port.
3. Start the measurement.
   After the sample run, the instrument stops and cleans automatically.
4. Remove the sample tube from the sample port and dispose of tube and remaining blood sample in accordance with local laboratory biohazard safety procedures.

Do not start a second measurement with the leftovers in the sample tube. If a duplicate or repeat measurement is required, use a new sample tube and prepare a fresh sample.

12.4 Data acquisition and analysis

The following steps describe the data acquisition and analysis on a suitable CyFlow™ Counter.

Data analysis with the CyFlow™ Counter should only be performed after the internal quality control of the instrument was successful.

Please refer to the IFU of the CyFlow™ Counter for start-up procedure and maintenance of the device, if necessary.

The CD4 T cell counting result will be displayed on the screen. If the histogram does not show a clear peak of the CD4 positive stained T cells, the sample preparation and measurement must be repeated.

Results will be incorrect if gate positioning is not done precisely.

Be careful not to gate monocytes as CD4 positive stained T cells if CD4 concentration is extremely low in a patient blood sample.

13 Calculation of examination results

Calculation of the cell concentration is part of the software-based data analysis, which provides the CD4 T cells per µL blood sample after each measurement. For further information, please refer to the IFU of the Sysmex Partec IVD flow cytometer, such as the CyFlow™ Counter.

14 Interpretation of results

Human Immunodeficiency Virus (HIV) is one of the main reasons for CD4 T cell depletion, which debilitates the immune system. The CD4 T cell concentration is useful to assess the immune and clinical status of patients. It is an indicator for the initiation or follow-up of treatment for people living with HIV, in conjunction with other laboratory and clinical findings. Results should be interpreted by physicians, in conjunction with locally applicable HIV treatment guidelines.

15 Analytical performance characteristics

15.1 Analytical sensitivity

Please refer to Limit of detection in section 15.4.

15.2 Analytical specificity

The clone MEM-241 recognizes CD4 antigen, a 55kDa transmembrane glycoprotein expressed on a subset of T lymphocytes ("helper" T cells) and on monocytes, tissue macrophages and granulocytes. HCDM (former HLDA VIII) Meeting, May 2006, Québec, Canada; WS Code M241 [1]

15.3 Accuracy

Trueness/Bias

EDTA whole blood specimens were stained with CD4 easy count kit and analysed on the CyFlow™ Counter (CyView™ 2.11) at three test sites (n = 1022 total). CD4 absolute values were compared with results from a BD FACSCalibur™ using BD Tritest™ or Multitest reagents and BD Trucount™ tubes and showed a mean bias of -13.06% (CD4 absolute, limits of agreement: -47.22%; +21.11%) between both methods.

Precision

Tab. 1: Evaluation of repeatability for the CD4 values of the CD4 easy count kit using low and normal blood controls.

Sample	N	CD4 value – Repeatability (SD)	CD4 value – Repeatability (%CV in %)
Low Blood Control (< 200 CD4 T cells/µL)	404	7.8	6.0
Normal Blood Control (≥ 200 CD4 T cells/µL)	394	48.8	5.0

SD = Standard Deviation %CV = Coefficient of Variation as a percentage

Tab. 2: Evaluation of reproducibility for the CD4 values of the CD4 easy count kit using low and normal blood controls.

Sample	N	CD4 value – Reproducibility (SD)	CD4 value – Reproducibility (%CV in %)
Low Blood Control (< 200 CD4 T cells/µL)	220	11.22	8.45
Normal Blood Control (≥ 200 CD4 T cells/µL)	220	52.13	5.20

SD = Standard Deviation %CV = Coefficient of Variation as a percentage

15.4 Detection capabilities

The evaluation for Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) was carried out according to the specifications in the guideline CLSI EP17-A2.

LoB: 5 CD4 T cells/µL

LoD: 11 CD4 T cells/µL

LoQ: 20 CD4 T cells/µL

15.5 Measuring range/Linearity

For CD4 by using the polynomial regression method according to CLSI EP06-A, the method has been demonstrated to be linear from 40 to 2500 CD4 T cells/µL, with higher order polynomials with significant nonlinear coefficients within 10% of the 1st order fit at > 200 CD4 T cells/µL and within ± 20 cells/µL at ≤ 200 CD4 T cells/µL in this interval.

15.6 Assay cut-offs

The cut-offs are defined by the World Health Organization (WHO) and were initially specified as 200 cells/µL, later as 350 cells/µL and 500 cells/µL.[2][3][4] For managing Advanced HIV Disease (AHD) a cut-off of 200 cells/µL was set.[5] The clinical parameters were determined using the 3 cut-offs.

16 Clinical performance characteristics

16.1 Diagnostic sensitivity & specificity, positive & negative predictive value, likelihood ratio

Tab. 3: Evaluation of diagnostic parameters of blood specimens from HIV positive patients and other patients undergoing routine CD4 T+ cell enumeration from three sites for the CD4 values of the CD4 easy count kit with three different cut-offs, reference method: BD FACSCalibur™, n = 1022.

Cut-Off [cells/µL]	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	PLR (95% CI)	NLR (95% CI)
200	98.8 (95.8 – 99.7)	96.0 (94.5 – 97.1)	83.0 (77.2 – 87.6)	99.8 (99.1 – 99.9)	24.82 (17.85 – 34.52)	0.01 (0.00 – 0.05)
350	97.5 (95.4 – 98.7)	87.2 (84.4 – 89.5)	81.0 (77.0 – 84.3)	98.5 (97.1 – 99.2)	7.62 (6.23 – 9.31)	0.03 (0.02 – 0.05)
500	99.7 (98.8 – 99.9)	78.4 (74.3 – 82.0)	85.9 (83.1 – 88.3)	99.4 (97.9 – 99.8)	4.62 (3.86 – 5.52)	0.00 (0.00 – 0.02)

CI = Confidence Interval PPV = Positive Predictive Value NPV = Negative Predictive Value
PLR = Positive Likelihood Ratio NLR = Negative Likelihood Ratio

16.2 Expected values in normal and affected populations

Expected CD4 counts in healthy individuals with no HIV, in HIV positive patients under treatment or with low viral load: > 500 CD4 T cells/µL.[4] Cut-off values for HIV positive patients < 500 / 350 / 200 CD4 T cells/µL (see section 15.6).

17 Limitations

Patient measurements of CD4 and CD4% made using Sysmex Partec assays should not be used alongside measurements obtained from other manufacturer's methods of determining CD4 and CD4%. Use the assay only with a suitable Sysmex Partec IVD flow cytometer. Values obtained using other equipment or assays are not interchangeable.

Different endogenous and exogenous substances were tested according to the CLSI EP07-A2 guideline for possible interfering effects which could have an influence on the blood sample analysis. In summary, ≥ 18% haemolysis (induced by using the protocol recommended in the CLSI EP07-A2, Appendix G, G1 - Osmotic Shock Procedure) was observed to show an interfering effect. For that reason, haemolysed samples should be rejected. Excessive levels of white blood cells lead to increased CD4 concentrations.

The presence of antibodies in a patient's specimen (e.g., human anti-animal antibodies, rheumatoid factors, or therapeutic antibodies) can interfere with the principle of the examination procedure. If antibodies directly interfere with CD4 mAb PE, this could result in spuriously low CD4 concentrations.[6][7]

The presence of endogenous proteins in a patient's specimen (e.g., factors of the complement system or enzymes) can interfere with the principle of the examination procedure. If endogenous proteins directly interfere with CD4 mAb PE, this could result in spuriously low CD4 concentrations.[8]

Chronic smoking may lead to an increase in the number of leukocytes, which might lead to an over-estimation of cell counts.[9][10]

Accurate and reproducible results will be obtained if the procedures used are in accordance with the IFU and compatible with good laboratory practices. This includes the avoidance of contaminations from various sources such as sample collection and preparation material.

18 Literature

[1] Zola et al. CD molecules 2006 – Human cell differentiation molecules. *Journal of Immunological Methods*, 2007; 319: 1-5.
[2] World Health Organization. *Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach*. Geneva: World Health Organization; 2006.
[3] World Health Organization. *Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach*. Geneva: World Health Organization; 2010.
[4] World Health Organization. *Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach*. Geneva: World Health Organization; 2013.
[5] World Health Organization. *Guidelines for Managing Advanced HIV Disease and Rapid Initiation of Antiretroviral Therapy*. Geneva: World Health Organization; 2017.
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[10] Pedersen et al. Smoking and increased white and red blood cells. A Mendelian randomization approach in the Copenhagen General Population Study. *Arterioscler Thromb Vasc Biol*, 2019; 39: 965-977. doi: 10.1161/ATVBAHA.118.312338

19 Summary of safety and performance

The summary of safety and performance will be supplied in the Eudamed database.

20 Manufacturer

Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz
Germany

Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com
www.sysmex-partec.com

21 Symbols

REF	Reference number		Manufacturer	LOT	Batch code
	Keep away from sunlight		Temperature limit	IVD	In vitro diagnostic medical device
	Use-by date		Consult instructions for use	CE	CE mark
Σ	Contains sufficient for <n> tests	CONTENT	Content of kit	UDI	Unique device identifier

22 Date of issue or revision

Rev.: 001 CN 2149
Rev. date: 13-03-2024
Doc. No.: 05-8410 IFU GB EN

1.3 CD4% easy count kit IFU (product code 05-8405)

IVD

Identification of the IVD reagent
Product name:
CD4% easy count kit
REF 05-8405

Content:
Vial containing CD4 mAb PE — REF 05-8405-01
Vial containing CD45 mAb PE-Cy5 — REF 05-8405-02
Bottle containing Buffer 1 — REF 05-8405-03
Bottle containing Buffer 2 — REF 05-8405-04

1 Specification

Specificity	Human CD4	Human CD45
Isotype	IgG1	IgG1
Content	100 tests	100 tests
Fluorochrome	PE	PE-Cy5
λ excitation (nm)	532 / 488	532 / 488
Emission maximum (nm)	578	670

2 Intended Use
IVD For In Vitro Diagnostic Use.
The CD4% easy count kit is a manual, four-component, quantitative IVD test for labelling of leukocytes and a subpopulation of lymphocytes in adult venous EDTA whole blood, which can then be enumerated with the Sysmex Partec CyFlow™ Counter IVD flow cytometer. The CD4 T cell concentration and CD4% of lymphocytes in blood samples are useful indicators for the initiation or follow-up of treatment for HIV positive patients in conjunction with other laboratory and clinical findings.
The test is intended to be performed by trained healthcare professionals.

3 Principle of the examination method
An aliquot of an EDTA whole-blood sample is mixed with two antibodies (CD4 and CD45), each conjugated to a different fluorochrome for labelling dedicated cell populations. After a fixed incubation time, the two buffer solutions are added and the sample is ready for analysis on the CyFlow™ Counter flow cytometer. The light source excites the fluorescent dye linked with the stained cell and the emitted light is detected while a precise volume of blood sample is running through the instrument. The concentration of the dedicated cell populations is calculated by the integrated software.
For further information, please refer to the IFU of CyFlow™ Counter (CY-S-3022IFUEN).

4 Storage and shelf life after first opening
1. Storage:
Store the antibody and buffer reagents at 2 - 8°C in the dark. Do not freeze or expose the reagents to elevated temperatures and keep them away from direct sunlight.
2. Shelf life after first opening:
Always close the bottle after use and use new pipette tips each time the reagent is sampled to avoid contamination.
Under these storage conditions, the CD4% easy count kit will be stable until the expiration date printed on the kit label.

5 Components
CD4 mAb PE and CD45 mAb PE-Cy5 are murine monoclonal antibodies supplied in PBS buffer with 0.2% BSA and 0.09% sodium azide. Buffer 1 and Buffer 2 are PBS-based solutions containing 0.09% sodium azide.

6 Evidence of deterioration
The antibody and buffer solutions are clear liquids. Do not use the reagents if there is the appearance of any kind of turbidity or contamination.
For questions regarding the performance or quality of the product, please contact your local Sysmex representative.

7 Precaution and warnings
Reagents contain 14 mM sodium azide as a preservative. The low concentration of sodium azide does not require hazard labelling, but the normal safety precautions for the handling of chemicals must be observed. Refer to the Safety Data Sheet (SDS) for a complete list of warnings and precautions.

8 Additional required equipment

Instrument requirement:	CyFlow™ Counter (REF No.: CY-S-3022)
Required apparatus:	Sample Tubes 3.5 ml (REF No.: 04-2000) A verified pipette 10 µl and pipette tips A verified pipette 20 µl and pipette tips A verified pipette 100 – 1000 µl and pipette tips Venous blood collection system with EDTA as anticoagulant Stop watch

9 Reagent preparation
CD4 mAb PE (REF No.: 05-8405-01), the antibody reagent is ready to use.
CD45 mAb PE-Cy5 (REF No.: 05-8405-02), the antibody reagent is ready to use.
Buffer 1 (REF No.: 05-8405-03), the buffer solution is ready to use.
Buffer 2 (REF No.: 05-8405-04), the buffer solution is ready to use.

10 Primary sample collection, handling and storage
WARNING ⚠ *All biological specimens and material are considered as biohazards and should be handled as if capable of transmitting infection. Appropriate safety precautions and handling procedures must be applied in accordance with applicable laws and regulations.*

- The blood sample must be a venous, whole-blood sample, collected with a blood collection system which contains EDTA as anticoagulant.
- The blood sample must be transported in a dark container, protected from light and not exposed to elevated temperatures. A suitable transport container is, for example, a Styrofoam box with cooling material. Best transport conditions would be below 25 °C if transport will not take more than 6 hours after donation.
- For transport and storage time more than 6 hours, blood must be kept at 2 - 8 °C.
- Do not freeze and thaw the blood sample.
- For best conditions, the blood sample should be fresh, i.e. not more than six hours should have elapsed between sample collection and analysis.
- Blood samples can be used up to 24 hours after drawing if they are stored in the fridge at 2 - 8 °C.
- Before use, the sample tubes containing the blood must be inverted gently 8 to 10 times.
- Do not use clotted blood samples.

11 Examination procedure
NOTICE *Reverse pipetting is critical to accuracy especially when dispensing viscous and very small sample volumes. For whole blood pipetting, we recommend using a calibrated electronic pipette which is preprogrammed to operate in the reverse pipetting mode.*
If an electronic pipette is not available, follow these instructions for manual reverse pipetting:
- *Depress the operating button to the second stop. Let the operating button move up completely, excess sample is drawn up into the tip.*
- *Depress the operating button to the first stop to expel a precise volume of blood, leaving excess sample in the tip.*
- *Discard pipette tip with excess blood sample in the tip into medical waste container.*

Please note, you must not use reverse pipetting for antibody or buffer solutions.

11.1 Staining
- Invert the blood sample in the EDTA blood collection tube gently 8 to 10 times.
- Pipette 20 µl EDTA whole blood sample to the bottom of a sample tube, avoid leaving a trail of blood on the inner tube wall from pipette tip. Discard the pipette tip.
- Add 10 µl CD4 mAb PE directly into the blood sample and mix it gently, avoid leaving a trail of blood on the inner tube wall from pipette tip. Discard the pipette tip.
- Add 10 µl CD45 mAb PE-Cy5 directly into the blood sample and mix it gently, avoid leaving a trail of blood on the inner tube wall from pipette tip. Discard the pipette tip.
- Incubate the mixture for 15 minutes at 15 - 30 °C in the dark.
- Add 400 µl Buffer 1 and vortex briefly, or tap tube gently in order to mix the sample. Discard the pipette tip.
- Add 400 µl Buffer 2 and vortex briefly, or tap tube gently in order to mix the sample. Discard the pipette tip.
- Vortex briefly, or tab tube gently before analysing with the CyFlow™ Counter.

NOTICE *After addition of Buffer 1, samples can be stored for up to 2 hours at 2 - 8 °C in the dark. Do not use the sample later than that or if not prepared accordingly.*
After addition of Buffer 2, samples must be analysed within 10 minutes.

11.2 Sample analysis
Please refer to the IFU of the CyFlow™ Counter (CY-S-3022IFUEN) for how to operate the instrument before analysing the sample. The start-up procedures and the internal quality control procedure must be successfully completed before sample analysis.
- Choose and load the configuration for CD4% measurement from the menu bar of CyFlow™ Counter.
- Insert the sample tube with the prepared blood sample into the sample port.
- Start measurement.
- After sample run, the instrument stops and cleans automatically.
- Remove and dispose of blood sample in accordance with local laboratory biohazard safety procedures.

Do not start a second measurement with the same sample tube. If a duplicate or repeat measurement is required, a second sample tube has to be prepared as per 11.1.

11.3 Data acquisition and analysis
Data analysis with the CyFlow™ Counter is only possible if the internal quality control of the instrument was successful.
Please refer to the IFU of the CyFlow™ Counter (CY-S-3022IFUEN) for start-up procedure and maintenance of the device, if necessary.
The CD4 T-cell counting result, CD4 percentage and total count of lymphocytes will be displayed on the screen. For results to be reliable there must be good separation of cell clusters as shown in Figure 1. If the clusters of stained cells towards the debris signals in the CD45 – SSC dot plot are not clearly separable (see Figure 2), or the clusters of CD4 positive cells, CD4 negative cells and monocytes in the CD4 – SSC dot plot are not clearly separable, the sample preparation and measurement must be repeated.

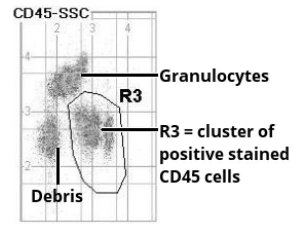

Figure 1: Example of well separated clusters of granulocytes, other CD45 positive stained leucocytes and debris signals.

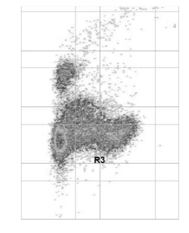
Figure 2: Example of bad separation between debris and CD45 positive stained leucocytes. This sample is not analysable.

Results will be incorrect if gate positioning of CD45 positive stained cells (R3) is not done precisely.

12 Calculation of examination results
Calculation of the cell concentration is part of the software-based data analysis, which provides the result in CD4%, and CD4+ cells per µl blood sample after each measurement. For further information, please refer to the IFU of the CyFlow™ Counter (CY-S-3022IFUEN).

13 Interpretation of results
Human Immunodeficiency Virus (HIV) is one of the main reasons for CD4 cell depletion, which debilates the immune system. Results should be interpreted by physicians, in conjunction with locally applicable HIV treatment guidelines.

14 Control procedure
Appropriate quality control should be performed according to local and national regulations. A variety of stabilized blood samples are available on the market, only a few are suitable when using certain flow cytometers. If the acceptance criterion of the individual control material is not met, do not proceed with patient specimen testing and contact your local Sysmex representative. For further information about the use of adequate control material, please contact your local Sysmex representative.

15 Performance characteristics
15.1 Specificity
The antibody MEM-241 recognizes CD4 antigen, a 55 kDa transmembrane glycoprotein expressed on a subset of T lymphocytes ("helper" T-cells) and on monocytes, tissue macrophages and granulocytes. HCDM (former HLDA VIII) Meeting, May 2006, Québec, Canada; WS Code M241
The antibody MEM-28 reacts with all alternative forms of human CD45 antigen (Leukocyte Common Antigen), a 180-220 kDa single chain type I transmembrane protein expressed at high level on all cells of hematopoietic origin, except erythrocytes and platelets. HLDA III; WS Code NL 833a

15.2 Detection ability
The evaluation for Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) was carried out according to the specifications in the guideline CLSI EP17-A2.
LoB: ≤50% of LoD
LoD: ≤20 CD4 cells/µl
LoQ: ≤40 CD4 cells/µl

15.3 Repeatability
Intra- and inter-assay variation:
CV values are less than 10% for CD4 values of 200 CD4 T-cells/µl or above.
CV values are less than 10% for CD4% values for 25% or above.
CV values are less than 15% for CD4 absolute values below 200 CD4 T-cells/µl.
SD values are less or equal to 2.5% for CD4% values below 25%.

15.4 Linearity
The measuring range of the assay and linearity of the measurement are valid over the range:
CD4 absolute: 40-2500 CD4 T-cells/µl
CD4%: 4 – 60%

15.5 Trueness
EDTA whole blood specimen were stained with CD4% easy count kit and analysed on the CyFlow™ Counter (CyView™ 2.11). CD4 absolute and CD4% values were compared with results from a BD FACSCalibur using BD Tritest CD3/CD4/CD45 and BD Trucount tubes and showed a bias of -22.56% (CD4 absolute) and +2.1% (CD4%) between both methods.

16 Limitations
CAUTION ⚠ *Sysmex Partec CD4% easy count kits (05-8405) have not been validated for use with pediatric and adolescent patients.*

CAUTION ⚠ *Patient measurements of CD4 and CD4% made using Sysmex Partec assays should not be used alongside measurements obtained from other manufacturer's methods of determining CD4 and CD4%. Use the assays only with Sysmex Partec instruments. Values obtained using other equipment or assays are not interchangeable.*

Different endogenous and exogenous substances were tested regarding the CLSI EP07-A2 guideline for possible interfering effects which could have an influence to the blood sample analysis.
Finally, ≥ 18% haemolysis (induced by using the protocol recommended in the CLSI EP07-A2, Appendix G, G1 - Osmotic Shock Procedure) was observed to show an interfering effect. For that reason, haemolyzed samples should be rejected. For CD4 and CD4% determination in whole blood samples with CyFlow™ Counter assays, no other anticoagulant than EDTA should be used.
For further questions regarding the performance of the product, please contact your local Sysmex representative.

17 Literature references

1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
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5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Contact of manufacturer

Manufacturer
Sysmex Partec GmbH
Arndtstraße 11 a-b
02828 Görlitz, Germany
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Version number of IFU and date of issue

Revision: Rev-07_10-09-2020_CN 1563
Issued by: Sysmex Partec GmbH

Safety Data Sheet for this product is available at www.sysmex-partec.com/services.
Cy and CyDye are trademarks of GE Healthcare.

Identifikation des IVD-Reagens
Produktname:
CD4% easy count kit
REF 05-8405

Inhalt:
Ampulle mit CD4 mAb PE — REF 05-8405-01
Ampulle mit CD45 mAb PE-Cy5 — REF 05-8405-02
Flasche mit Puffer 1 — REF 05-8405-03
Flasche mit Puffer 2 — REF 05-8405-04

1 Specification

Spezifität	Humanes CD4	Humanes CD45
Isotyp	IgG1	IgG1
Inhalt	100 Tests	100 Tests
Fluorochrom	PE	PE-Cy5
λ Anregung (nm)	532 / 488	532 / 488
Emissionsmaximum (nm)	578	670

2 Zweckbestimmung
IVD In-vitro-Diagnostikum.
Das **CD4% easy count kit** ist ein manueller, quantitativer 4-Komponenten-In-vitro-Test für die Markierung von Leukozyten und einer Lymphozyten-Untergruppe in EDTA-Vollblutproben von Erwachsenen, die anschließend mit dem Sysmex Partec IVD-Flowzytometer CyFlow™ Counter ausgezählt werden können. Die Konzentration der CD4-positiven T-Helferzellen sowie der Anteil der CD4-Helferzellen an den Lymphozyten (CD4%) in Blutproben sind in Verbindung mit anderen Laborwerten und klinischen Befunden nützliche Indikatoren für die Einleitung der Behandlung HIV-positiver Patienten und die Nachsorge.
Der Test ist nur von geschulten medizinischen Fachkräften durchzuführen.

3 Prinzip des Untersuchungsverfahrens
Ein Aliquot einer EDTA-Vollblutprobe wird mit zwei Antikörpern gemischt (CD4 und CD45), wobei jeder mit einem anderen Fluorochrom zur Markierung von spezifischen Zellpopulationen konjugiert ist. Nach einer bestimmten Inkubationszeit werden die beiden Pufferlösungen hinzugegeben; nun kann die Analyse der Probe im CyFlow™ Counter-Flowzytometer erfolgen. Die Lichtquelle regt den Fluoreszenzfarbstoff an, der mit der gefärbten Zelle verbunden ist, und das emittierte Licht wird detektiert, während ein genaues Volumen der Blutprobe durch das Instrument läuft. Die Konzentration der spezifischen Zellpopulationen wird von der integrierten Software berechnet.
Weitere Informationen entnehmen Sie bitte dem Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFU-EN).

4 Lagerung und Haltbarkeitsdauer nach dem ersten Öffnen
1. Lagerung:
Das Antikörper- und Pufferreagens bei 2 - 8 °C im Dunkeln aufbewahren. Nicht einfrieren oder erhöhten Temperaturen aussetzen. Vor direkter Sonneneinstrahlung schützen
2. Haltbarkeitsdauer nach dem ersten Öffnen:
Die Flasche nach jedem Gebrauch wieder verschließen und für jede weitere Entnahme des Reagens neue Pipettenspitzen verwenden, um eine Verunreinigung zu vermeiden.
Unter Einhaltung der oben genannten Lagerbedingungen ist das CD4% easy count kit bis zum Verfallsdatum auf dem Etikett des Kits stabil.

5 Bestandteile
CD4 mAb PE und CD45 mAb PE-Cy5 sind murine monoklonale Antikörper in PBS-Puffer mit 0,2 % BSA und 0,09 % Natriumazid. Puffer 1 und Puffer 2 sind Lösungen auf PBS-Basis, die 0,09 % Natriumazid enthalten.

6 Anzeichen von Verderb
Die Antikörper- und Pufferlösungen sind klare Flüssigkeiten. Die Reagenzien nicht verwenden, falls Trübungen oder Verunreinigungen auftreten. Bei Fragen zur Leistung oder Qualität des Produkts wenden Sie sich bitte an Ihre nächstgelegene Sysmex Vertretung.

7 Vorsichtsmaßnahmen und Warnhinweise
Die Reagenzien enthalten 14 mM Natriumazid als Konservierungsstoff. Aufgrund der niedrigen Natriumazid Konzentration ist eine Gefahrstoffkennzeichnung nicht erforderlich, jedoch sind die beim Umgang mit Chemikalien üblichen Vorsichtsmaßregeln zu beachten. Beachten Sie das Sicherheitsdatenblatt (SDB) für eine vollständige Liste von Warnhinweise und Vorsichtsmaßnahmen.

8 Zusätzlich erforderliche Ausrüstung

Erforderliche Instrumente:	CyFlow™ Counter (REF No.: CY-S-3022)
Benötigte Ausrüstung:	Probenröhrchen 3.5 ml (Code No.: 04-2000) Eine verifizierte 10-µl-Pipette und Pipettenspitzen Eine verifizierte 20-µl-Pipette und Pipettenspitzen Eine verifizierte 100- bis 1000-µl-Pipette und Pipettenspitzen System zur venösen Blutentnahme mit EDTA als Antikoagulans Stoppuhr

9 Reagent preparation
CD4 mAb PE (REF No.: 05-8405-01), das Antikörperreagens ist gebrauchsfertig.
CD45 mAb PE-Cy5 (REF No.: 05-8405-02), das Antikörperreagens ist gebrauchsfertig.
Puffer 1 (REF No.: 05-8405-03), die Pufferlösung ist gebrauchsfertig.
Puffer 2 (REF No.: 05-8405-04), die Pufferlösung ist gebrauchsfertig.

10 Entnahme, Handhabung und Lagerung der Primärprobe
WARNUNG ⚠ *Alle biologischen Proben und Materialien sind als gefährliches biologisches Material und als potenziell infektiös zu behandeln. Es sind angemessene Sicherheitsvorkehrungen und Verfahren zur Handhabung gemäß den geltenden Gesetzen und Vorschriften anzuwenden.*

- Bei der Blutprobe muss es sich um eine venöse Vollblut-Probe handeln, die mithilfe eines Blutentnahmesystems entnommen wurde, das EDTA als Antikoagulans verwendet.
- Die Blutprobe ist in einem dunklen, vor Licht geschützten Behälter zu transportieren und darf nicht erhöhten Temperaturen ausgesetzt werden. Ein geeigneter Transportbehälter ist z. B. eine Styroporbox mit Kühlung. Die besten Transportbedingungen würden unter 25 °C liegen, wenn der Transport nicht länger als 6 Stunden nach der Spende dauert.
- Für eine Transport- und Lagerzeit von mehr als 6 Stunden muss das Blut bei 2 - 8 °C aufbewahrt werden
- Die Blutprobe nicht einfrieren und wieder auftauen.
- Optimale Bedingungen sind bei Verwendung einer frischen Blutprobe gegeben, d. h. es sollten nicht mehr als sechs Stunden zwischen der Blutentnahme und der Analyse liegen.
- Bei Lagerung im Kühlschrank bei 2 - 8 °C können Blutproben bis zu 24 Stunden nach der Entnahme verwendet werden.
- Vor der Anwendung die Probenröhrchen 8 - 10 mal vorsichtig umdrehen.
- Keine geronnenen Blutproben verwenden.

11 Untersuchungsverfahren
HINWEIS *Reverses Pipettieren ist entscheidend für die Genauigkeit, insbesondere bei der Abgabe viskoser und sehr kleiner Probenmengen. Für das Pipettieren von Vollblut empfehlen wir die Verwendung einer kalibrierten elektronischen Pipette, die für den Betrieb im reversen Pipettiermodus vorprogrammiert ist.*
Steht keine elektronische Pipette zur Verfügung, führen Sie das reverse Pipettieren gemäß den folgenden Anweisungen manuell durch:
- *Bedienknopf bis zum zweiten Anschlag herunterdrücken. Bedienknopf vollständig zurückgleiten lassen. Es wird eine größere Menge Probe in die Spitze aufgezogen.*
- *Bedienknopf bis zum ersten Anschlag herunterdrücken, um ein genaues Blutvolumen abzugeben. Anschließend befindet sich noch ein Rest der Probe in der Spitze.*
- *Pipettenspitze mit dem verbliebenen Rest der Blutprobe in einen Behälter für medizinische Abfälle entsorgen.*

Beachten Sie bitte, dass reverses Pipettieren nicht für Antikörper- oder Pufferlösungen eingesetzt werden darf.

11.1 Markierung
- Die Blutprobe im EDTA-Blutentnahmeröhrchen 8 - 10 mal vorsichtig umdrehen.
- 20 µl EDTA-Vollblutprobe auf den Boden eines Probenröhrchens pipettieren. Dabei keine Blutspuren von der Pipettenspitze auf der Röhrcheninnenwand hinterlassen. Pipettenspitze entsorgen.
- 10 µl CD4 mAb PE direkt in die Blutprobe geben und vorsichtig mischen. Dabei keine Blutspuren von der Pipettenspitze auf der Röhrcheninnenwand hinterlassen. Pipettenspitze entsorgen.
- 10 µl CD45 mAb PE-Cy5 direkt in die Blutprobe geben und vorsichtig mischen. Dabei keine Blutspuren von der Pipettenspitze auf der Röhrcheninnenwand hinterlassen. Pipettenspitze entsorgen.
- Inkubieren Sie die Mischung 15 Minuten bei 15 - 30 °C im Dunkeln.
- 400 µl Puffer 1 hinzugeben und kurz vortexen oder das Röhrchen sanft aufklopfen, um die Probe zu mischen. Pipettenspitze entsorgen.
- 400 µl Puffer 2 hinzugeben und kurz vortexen oder das Röhrchen sanft aufklopfen, um die Probe zu mischen. Pipettenspitze entsorgen.
- Vor der Analyse mit dem CyFlow™ Counter kurz vortexen oder das Röhrchen sanft aufklopfen.

HINWEIS *Nach der Zugabe von Puffer 1 können die Proben bis zu 2 Stunden bei 2 - 8 °C im Dunkeln aufbewahrt werden. Die Probe danach nicht mehr verwenden und auch dann nicht, wenn sie nicht wie angegeben vorbereitet wurde.*
Nach der Zugabe von Puffer 2 müssen die Proben innerhalb von 10 Minuten analysiert werden.

11.2 Probenanalyse
Bitte vor der Probenanalyse das Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFUEN) für die Verwendung des Geräts beachten. Vor der Probenanalyse müssen die Verfahren zur Inbetriebnahme und internen Qualitätskontrolle erfolgreich abgeschlossen worden sein.
- Wählen und laden Sie die Konfiguration für die CD4% Messung aus der Menüleiste des CyFlow™ Counter.
- Das Probenröhrchen mit dem aufbereiteten Blut in den Probenport setzen.
- Die Messung starten.
- Nach dem Probendurchlauf stoppt das Instrument und wird automatisch gereinigt.
- Die Blutprobe entnehmen und gemäß den örtlichen Sicherheitsvorschriften in Bezug auf biogefährliche Materialien in Labors entsorgen.

Keine erneute Messung mit demselben Probenröhrchen durchführen. Wenn eine Zweifachmessung oder eine Wiederholung der Messung erforderlich ist, muss ein zweites Probenröhrchen wie unter 11.1 beschrieben vorbereitet werden.

11.3 Datenerfassung und -analyse
Die Datenanalyse kann erst erfolgen, wenn die interne Qualitätskontrolle des CyFlow™ Counter erfolgreich abgeschlossen wurde. Im Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFUEN) finden Sie eine Beschreibung der Inbetriebnahme und Wartung des Geräts. Das CD4-T-Zell-Zählresultat, der CD4-Prozentsatz und die Gesamtzahl der Lymphozyten werden auf dem Bildschirm angezeigt. Wenn die Haufen der markierten Zellen im CD45 SSC-Dot Plot von Rauschsignalen nicht eindeutig abgrenzbar sind (siehe Abbildung 2) oder die Haufen CD4-positiver Zellen, CD4-negativer Zellen und Monozytenhaufen im CD4 SSC-Dot Plot nicht eindeutig voneinander abgrenzbar sind, muss die Probenvorbereitung und -messung wiederholt werden.

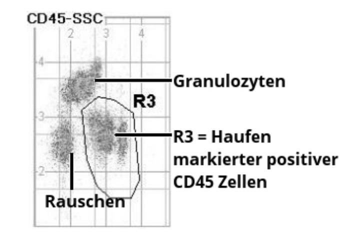

Abbildung 1: Beispiel für gute Abgrenzung von Granulozyten-Haufen, anderen markierten CD45-positiven Leukozyten und Rauschsignalen.

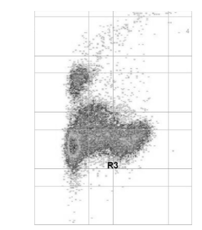
Abbildung 2: Beispiel für eine schlechte Trennung zwischen Rauschen und CD45-positiv gefärbten Leukozyten. Diese Probe ist nicht analysierbar.

Es kommt zu falschen Ergebnissen, wenn die Positionierung von CD45-positiv gefärbten Zellen (R3) nicht genau erfolgt.

12 Berechnung der Untersuchungsergebnisse
Die Berechnung der Zellkonzentration erfolgt im Rahmen der Software-basierten Datenanalyse. Die Ergebnisse werden nach jeder Messung als CD4%, CD4+-Zellen pro µl Blutprobe und Lymphozyten pro µl Blutprobe dargestellt.
Weitere Informationen entnehmen Sie bitte dem Benutzerhandbuch des CyFlow™ Counter (CY-S-3022IFU-EN).

13 Interpretation der Ergebnisse
Das Humane Immundefizienzvirus (HIV) ist einer der Hauptgründe für die CD4-Zelldepletion, die das Immunsystem schwächt. Die Ergebnisse sollten von Ärzten in Verbindung mit lokal anwendbaren HIV-Behandlungsrichtlinien interpretiert werden.

14 Kontrollverfahren
Es ist eine geeignete Qualitätskontrolle gemäß den einschlägigen lokalen und nationalen Vorschriften durchzuführen. Im Handel sind unterschiedliche Arten von stabilisierten Kontrollblutproben erhältlich. Nicht alle sind für jedes Flowzytometer geeignet.Welches Kontrollblut Sie verwenden können, erfahren Sie bei Ihrer Sysmex-Vertretung. Sind nach der Messung einer geeigneten Kontrollblutprobe die Akzeptanzkriterien nicht erfüllt, fahren Sie nicht fort mit der Analyse von Patientenproben.

15 Leistungsmerkmale
15.1 Spezifität
Der MEM-241-Antikörper erkennt das CD4-Antigen, ein transmembranes Glykoprotein mit einem Molekulargewicht von 55 kDa, das auf einer Untergruppe von T-Lymphozyten (T-Helferzellen) exprimiert wird, sowie auf Monozyten, Gewebsmakrophagen und Granulozyten. HCDM (ehemals HLDA VIII) Meeting, Mai 2006, Québec, Kanada; WS Code M241.
Der Antikörper MEM-28 reagiert mit allen Isoformen des humanen CD45-Antigens (Leukocyte Common Antigen), ein transmembranes Typ-I-Einzelkettenprotein mit einem Molekulargewicht von 180-220 kDa, das in hohem Maße auf allen Zellen hämatopoetischen Ursprungs, mit Ausnahme von Erythrozyten und Thrombozyten, exprimiert wird. HLDA III; WS Code NL 833a.

15.2 Nachweisfähigkeit
Die Leerwert-Obergrenze (Limit of Blank, LoB), die Nachweisgrenze (Limit of Detection, LoD) und die Quantifizierungsgrenze (Limit of Quantitation, LoQ) wurden gemäß den Spezifikationen der Richtlinie CLSI EP17-A2 ermittelt.
LoB: ≤50% of LoD
LoD: ≤20 CD4 Zellen/µl
LoQ: ≤40 CD4 Zellen/µl

15.3 Reproduzierbarkeit
Intra- und Inter-Assay-Varianz:
Die CV-Werte für CD4-Werte ≥ 200 CD4-T-Zellen/µl liegen unter 10 %.
Die CV-Werte für CD4%-Werte ≥ 25% liegen unter 10 %.
Die CV-Werte für absolute CD4-Werte < 200 CD4-T-Zellen/µl liegen unter 15 %.
Die Werte der Standardabweichung (SD) ≤ 2.5% für CD4% Werte liegen unter 25%.

15.4 Linearität
Der Messbereich des Assay und die Linearität der Messungen gelten für den folgenden Bereich:
Absoluter CD4 Wert: 40-2500 CD4 T-Zellen/µl
CD4%: 4 – 60%

15.5 Richtigkeit
EDTA-Vollblutproben wurden mit dem CD4% easy count kit markiert und im CyFlow™ Counter (CyView™ 2.11) analysiert. Die absoluten CD4-Werte und die CD4%-Werte wurden mit Ergebnissen eines BD FACSCalibur unter Verwendung von BD Tritest CD3/CD4/CD45 und BD Trucount-Röhrchen verglichen. Es zeigte sich eine Abweichung von -22,56 % (CD4 absolut) und +2,1 % (CD4%) zwischen den Methoden.

16 Grenzen des Verfahrens
ACHTUNG ⚠ *Die Sysmex Partec CD4% easy count kits (05-8405) wurden nicht für die Verwendung bei Kindern und Jugendlichen validiert.*

ACHTUNG ⚠ *Patientenmessungen von CD4 und CD4% mithilfe von Sysmex Partec Assays sollten nicht zusammen mit Methoden anderer Hersteller zur Bestimmung von CD4 und CD4% verwendet werden. Verwenden Sie die Assays nur mit Sysmex Partec Geräten. Werte, die mit anderen Geräten oder Assays erhalten wurden, sind nicht austauschbar.*

Verschiedene endogene und exogene Substanzen wurden in Übereinstimmung mit der Richtlinie CLSI EP07-A2 auf eine mögliche Beeinträchtigung der Blutprobenanalyse untersucht. Bei ≥ 18 % Hämolyse (gemäß dem in der Richtlinie CLSI EP07-A2, Anhang G empfohlenen Verfahren G1 „Osmotic Shock Procedure" induziert) wurde eine Beeinträchtigung beobachtet. Aus diesem Grund sollten hämolytische Proben verworfen werden. Für die Bestimmung von CD4 und CD4% in Vollblutproben mit CyFlow™ Counter Assays sollte kein anderes Antikoagulans als EDTA verwendet werden. Bei weiteren Fragen hinsichtlich des Leistung des Produkts wenden Sie sich bitte an Ihre nächstgelegene Sysmex-Vertretung.

17 Literatur

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18 Kontaktinformationen des Herstellers

Manufacturer
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Deutschland
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Versionsnummer des Benutzerhandbuchs und Ausgabedatum

Revision: Rev-07_10-09-2020_CN 1563
Issued by: Sysmex Partec GmbH

Das Sicherheitsdatenblatt zu diesem Produkt ist unter www.sysmex-partec.com/services erhältlich.
Cy und CyDye sind Marken der GE Healthcare.

Identification du réactif IVD
Nom du produit :
CD4% easy count kit
REF 05-8405

Contenu:
Fiole de CD4 mAb PE — REF 05-8405-01
Fiole de CD45 mAb PE-Cy5 — REF 05-8405-02
Flacon de tampon 1 — REF 05-8405-03
Flacon de tampon 2 — REF 05-8405-04

1 Spécification

Spécificité	CD4 humain	CD45 humain
Isotype	IgG1	IgG1
Contenu	100 tests	100 tests
Fluorochrome	PE	PE-Cy5
Excitation λ (nm)	532 / 488	532 / 488
Émission maximale(nm)	578	670

2 Usage prévu
IVD Pour la réalisation de diagnostics in vitro.
Le CD4% easy count kit est un test IVD quantitatif manuel constitué de quatre composants et destiné au marquage des leucocytes et d'une sous-population de lymphocytes dans le sang veineux total EDTA chez l'adulte, pouvant ensuite être dénombrés à l'aide du cytomètre en flux IVD CyFlow™ Counter de Sysmex Partec. La concentration en cellules T CD4 et le taux CD4% de lymphocytes dans les échantillons de sang sont des indicateurs utile pour l'initiation ou le suivi du traitement des porteurs du VIH, conjointement avec d'autres résultats cliniques et de laboratoire.
Le test doit être effectué par des professionnels de santé formés à cet effet.

3 Principe de la méthode d'examen
Une partie aliquote d'un échantillon de sang total EDTA est mélangée à deux anticorps (CD4 et CD45), conjugués chacun à un fluorochrome différent pour le marquage de populations cellulaires spécifiques. Après un temps d'incubation déterminé, les deux solutions tampons sont ajoutées et l'échantillon est prêt pour l'analyse sur un cytomètre en flux CyFlow™ Counter. La source de lumière excite le colorant fluorescent lié à la cellule colorée et la lumière émise est détectée alors qu'un volume précis d'échantillon de sang traverse l'instrument. Le logiciel intégré calcule la concentration des populations cellulaires spécifiques.
Pour de plus amples informations, se reporter au guide d'utilisation du CyFlow™ Counter (CY-S-3022IFU-EN).

4 Stockage et durée de conservation après la première ouverture
1. Stockage:
Stocker l'anticorps et les réactifs tampons à une température comprise entre 2 - 8 °C à l'abri de la lumière. Ne pas congeler ni exposer les réactifs à des températures élevées et les protéger du rayonnement solaire direct.
2. Durée de conservation après la première ouverture:
Toujours fermer le flacon après usage et utiliser de nouveaux embouts de pipettes à chaque fois qu'un échantillon de réactif est prélevé pour éviter toute contamination.
Dans ces conditions de stockage, le CD4% easy count kit restera stable jusqu'à la date de péremption imprimée sur l'étiquette du kit.

5 Composants
Le CD4 mAb PE et le CD45 mAb PE-Cy5 sont des anticorps monoclonaux murins fournis dans un tampon PBS avec 0,2 % de BSA et 0,09 % d'azoture de sodium. Le tampon 1 et le tampon 2 sont des solutions à base de PBS contenant 0,09 % d'azoture de sodium.

6 Signes de détérioration
La solution d'anticorps et la solution tampon sont des liquides clairs. Ne pas utiliser les réactifs en cas d'apparition d'un quelconque signe de turbidité ou de contamination. Pour toutes questions concernant la performance ou la qualité du produit, veuillez contacter votre représentant Sysmex local pour l'assistance technique.

7 Avertissements et mesures de précaution
Les réactifs contiennent de l'azoture de sodium 14 mM comme agent conservateur. La faible concentration de l'azoture de sodium ne nécessite aucun marquage comme substance dangereuse, mais les mesures de sécurité normales relatives à la manipulation des produits chimiques doivent être observées. Se reporter à la fiche de données de sécurité pour obtenir la liste complète des avertissements et mesures de précaution.

8 Équipement supplémentaire requis

Instruments requis:	CyFlow™ Counter (REF No.: CY-S-3022)
Matériel requis:	Tubes d'échantillon 3.5 ml (REF No.: 04-2000) Une pipette 10 µl validée et embouts de pipette Une pipette 20 µl validée et embouts de pipette Une pipette 100 – 1000 µl validée et embouts de pipette Système de prélèvement de sang veineux avec EDTA comme anticoagulant Chronomètre

9 Préparation des réactifs
CD4 mAb PE (REF No.: 05-8405-01), le réactif à base d'anticorps est prêt à l'emploi.
CD45 mAb PE-Cy5 (REF No.: 05-8405-02), le réactif à base d'anticorps est prêt à l'emploi.
Tampon 1 (REF No.: 05-8405-03), la solution tampon est prête à l'emploi.
Tampon 2 (REF No.: 05-8405-04), la solution tampon est prête à l'emploi.

10 Prélèvement, manipulation et stockage de l'échantillon primaire
AVERTISSEMENT ⚠ *Tous les échantillons et matériaux biologiques sont considérés comme biologiquement dangereux et doivent être manipulés comme s'ils étaient susceptibles de transmettre une infection. Les mesures de sécurité et les procédures de manipulation appropriées doivent être appliquées conformément aux lois et aux réglementations en vigueur.*

- L'échantillon de sang doit être un échantillon de sang veineux total, prélevé à l'aide d'un système de prélèvement sanguin contenant de l'EDTA comme anticoagulant.
- L'échantillon de sang doit être transporté dans un récipient sombre, hermétique à la lumière, et ne doit pas être exposé à des températures élevées. Une boîte en polystyrène avec un dispositif de réfrigération, par exemple, constitue un conteneur de transport approprié. Les meilleures conditions de transport seraient en dessous de 25 °C si le transport ne prend pas plus de 6 heures après le don.
- Pour le transport et le stockage plus de 6 heures, le sang doit être conservé entre 2 - 8 °C.
- Ne pas congeler ni décongeler l'échantillon de sang.
- Pour des conditions optimales, l'échantillon de sang doit être frais, c'est-à-dire qu'il ne doit pas s'être écoulé plus de six heures entre le prélèvement de l'échantillon et l'analyse.
- Les échantillons de sang peuvent être utilisés jusqu'à 24 heures après la prise de sang, s'ils sont conservés au réfrigérateur à une température comprise entre 2 - 8 °C.
- Avant l'utilisation, les tubes d'échantillon de sang doivent être retournés doucement 8 - 10 fois.
- Ne pas utiliser des échantillons de sang coagulé.

11 Procédure d'examen
REMARQUE *Le pipettage à l'envers est essentiel à la précision, tout particulièrement en cas de délivrance d'échantillons visqueux et de volumes d'échantillons très réduits. Pour le pipettage de sang total, nous recommandons l'utilisation d'une pipette électronique calibrée qui est préprogrammée pour fonctionner en mode de pipettage à l'envers.*
En l'absence d'une pipette électronique, suivre les présentes consignes pour procéder à un pipettage manuel à l'envers :
- *Enfoncer le bouton d'actionnement jusqu'à la deuxième butée. Laisser le bouton remonter entièrement, l'excès d'échantillon est aspiré dans la pointe.*
- *Enfoncer le bouton d'actionnement jusqu'à la première butée pour expulser un volume précis de sang tout en conservant l'excès d'échantillon dans la pointe.*
- *Éliminer la pointe de pipette contenant l'excès d'échantillon de sang dans une boîte à déchets médicaux.*

Prière de noter qu'il n'est pas nécessaire de procéder au pipettage à l'envers pour les anticorps ou les solutions tampons.

11.1 Marquage
- Renverser doucement l'échantillon de sang dans le tube de prélèvement de sang EDTA 8 - 10 fois.
- Pipeter 20 µl d'échantillon de sang total EDTA dans le fond d'un tube d'échantillon, en évitant de laisser un filet de sang sur la paroi intérieure du tube avec l'embout de pipette. Jetez l'embout de la pipette.
- Ajouter 10 µl de CD4 mAb PE directement dans l'échantillon de sang et mélanger doucement en évitant de laisser un filet de sang sur la paroi intérieure du tube avec l'embout de pipette. Jetez l'embout de la pipette.
- Ajouter 10 µl de CD45 mAb PE-Cy5 directement dans l'échantillon de sang et mélanger doucement en évitant de laisser un filet de sang sur la paroi intérieure du tube avec l'embout de pipette. Jetez l'embout de la pipette.
- Incuber le mélange pendant 15 minutes entre 15 - 30 °C et à l'abri de la lumière.
- Ajouter 400 µl de tampon 1 et mélanger brièvement au vortex ou tapoter doucement le tube afin de mélanger l'échantillon. Jetez l'embout de la pipette.
- Ajouter 400 µl de tampon 2 et mélanger brièvement au vortex ou tapoter doucement le tube afin de mélanger l'échantillon. Jetez l'embout de la pipette.
- Mélanger brièvement au vortex ou tapoter doucement le tube avant d'effectuer l'analyse à l'aide du CyFlow™ Counter.

REMARQUE *Après l'ajout du tampon 1, il est possible de conserver les échantillons jusqu'à 2 heures à une température comprise entre 2 - 8 °C à l'abri de la lumière. Ne pas utiliser l'échantillon après ce délai ou s'il n'a pas été préparé de manière conforme.*
Les échantillons doivent être analysés dans un délai de 10 minutes après l'ajout du tampon 2.

11.2 Analyse de l'échantillon
Pour obtenir des informations sur le fonctionnement de l'instrument avant l'analyse de l'échantillon, se reporter au guide d'utilisation du CyFlow™ Counter (CY-S-3022IFUEN). Les procédures de démarrage et la procédure de contrôle qualité interne doivent avoir été effectuées avec succès avant l'analyse de l'échantillon.
- Choisissez et chargez la configuration pour la mesure CD4% dans la barre de menu de CyFlow™ Counter.
- Insérer le tube d'échantillon avec l'échantillon de sang préparé dans l'orifice d'échantillonnage.
- Démarrer la mesure.
- Après l'analyse de l'échantillon, l'instrument s'arrête et se nettoie automatiquement.
- Retirer et éliminer l'échantillon de sang conformément aux procédures locales de sécurité en laboratoire relatives aux risques biologiques.

Ne pas démarrer de seconde mesure avec le même tube d'échantillon. S'il faut répéter ou effectuer deux fois la mesure, préparer un second tube d'échantillon conformément au point 11.1.

11.3 Acquisition et analyse des données
L'analyse des données à l'aide du CyFlow™ Counter n'est possible que si le contrôle qualité interne de l'instrument a été effectué avec succès. Consulter le guide d'utilisation du CyFlow™ Counter (CY-S-3022IFU-EN) pour effectuer une procédure de démarrage et la maintenance de l'appareil, si nécessaire.
Le résultat du comptage des cellules T CD4, le taux de pourcentage CD4 et la quantité totale de lymphocytes s'affichent à l'écran. Si les amas de cellules marquées ne sont pas clairement différenciables des signaux de bruit de fond dans le dot plot CD45 – SSC (voir Figure 2), ou si les amas de cellules positives CD4, de cellules négatives CD4 et de monocytes ne sont pas clairement différenciables dans le dot plot CD4 – SSC, il faut recommencer la préparation et la mesure de l'échantillon.

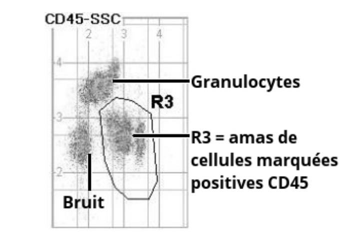

Figure 1: Exemple de bonne différenciation des amas de granulocytes, d'autres leucocytes marqués positifs CD45 et des signaux de bruit.

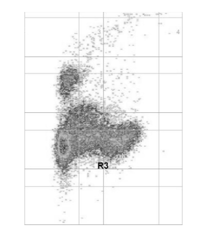
Figure 2: Exemple de mauvaise séparation entre les signaux de bruit et les leucocytes colorés CD45. Cet échantillon n'est pas analysable.

Les résultats seront incorrects si le positionnement de la porte des cellules colorées positives CD45 (R3) n'est pas fait avec précision.

12 Calcul des résultats d'examen
Le calcul de la concentration cellulaire fait partie intégrante de l'analyse des données par logiciel qui fournit le résultat en CD4%, en cellules CD4+ par µl d'échantillon de sang et en lymphocytes par µl d'échantillon de sang, après chaque mesure.
Pour de plus amples informations, se reporter au guide d'utilisation du CyFlow™ Counter (CY-S-3022IFUEN).

13 Interprétation des résultats
Le virus d'immunodéficience humaine (VIH) est l'une des principales raisons de la déplétion des cellules CD4, ce qui affaiblit le système immunitaire. Les résultats doivent être interprétés par les médecins, en conjonction avec les directives de traitement du VIH applicables localement.

14 Procédure de contrôle
Un contrôle qualité approprié doit être effectué conformément aux réglementations locales et nationales. Il existe différents types d'échantillons de sang de contrôle stabilisé disponibles sur le marché de détail. Tous ne conviennent pas à chaque cytomètre en flux. Vous pouvez trouver le sang de contrôle que vous utilisez à votre représentant Sysmex. Si les critères d'acceptation ne sont pas satisfaits après la mesure d'un échantillon de sang de contrôle approprié, ne pas procéder à l'analyse des échantillons de patients.

15 Caractéristiques de performance
15.1 Spécificité
L'anticorps MEM-241 reconnaît l'antigène CD4, une glycoprotéine transmembranaire 55 kDa exprimée sur une sous-population de lymphocytes T (cellules T « auxiliaires »), ainsi que sur les monocytes, les macrophages tissulaires et les granulocytes. HCDM (former HLDA VIII) Meeting, mai 2006, Québec, Canada ; WS Code M241
L'anticorps MEM-28 réagit avec toutes les formes alternatives de l'antigène CD45 humain (Leukocyte Common Antigen), une protéine transmembranaire à chaîne unique de type I 180-220 kDa exprimée en forte densité sur toutes les cellules d'origine hématopoïétique, à l'exception des érythrocytes et des plaquettes. HLDA III ; WS Code NL 833a

15.2 Capacité de détection
Les évaluations de la limite du blanc (LoB), de la limite de détection (LoD) et de la limite de quantification (LoQ) ont été réalisées conformément aux spécifications de la directive CLSI EP17-A2.
LoB : ≤ 50 % de la LoD
LoD : ≤ 20 cellules CD4/µl
LoQ : ≤ 40 cellules CD4/µl

15.3 Répétabilité
Variation intra- et inter-essai :
Les valeurs du coefficient de variation (CV) sont inférieures à 10 % pour des valeurs de CD4 supérieures ou égales à 200 cellules T CD4/µl.
Les valeurs du coefficient de variation (CV) sont inférieures à 10 % pour des valeurs de CD4% supérieures ou égales à 25 %.
Les valeurs du coefficient de variation (CV) sont inférieures à 15 % pour des valeurs absolues de CD4 inférieures à 200 cellules T CD4/µl.
Les valeurs de l'écart-type (SD) ≤ 2,5% pour les valeurs CD4% sont inférieures à 25%.

15.4 Linéarité
La plage de mesure de l'essai et la linéarité de la mesure sont valides sur la plage :
taux absolu de CD4 : 40-2500 cellules T CD4/µl
CD4% : 4 – 60%

15.5 Justesse
Les échantillons de sang total EDTA ont été marqués avec le CD4% easy count kit et analysés sur le CyFlow™ Counter (CyView™ 2.11). Les valeurs absolues de CD4 et les valeurs de CD4% ont été comparées aux résultats obtenus à l'aide d'un BD FACSCalibur en utilisant des réactifs BD Tritest CD3/CD4/CD45 et des tubes BD Trucount, ce qui a révélé des écarts de -22,56 % (CD4 absolu) et +2,1 % (CD4%) entre les deux méthodes.

16 Limites de la procédure
PRUDENCE ⚠ *L'utilisation des kits CD4% easy count (05-8405) Sysmex Partec n'a pas été validée chez les patients pédiatriques et adolescents.*

PRUDENCE ⚠ *Les mesures des patients de CD4 et de CD4% effectuées à l'aide des tests Sysmex Partec ne doivent pas être utilisées avec les mesures obtenues à partir des méthodes de détermination des CD4 et CD4% d'autres fabricants. Utilisez les tests uniquement avec les instruments Sysmex Partec. Les valeurs obtenues avec d'autres équipements ou dosages ne sont pas interchangeables.*

Différentes substances endogènes et exogènes ont été testées conformément à la directive CLSI EP07-A2 pour vérifier les éventuelles interférences pouvant avoir une influence sur l'analyse de l'échantillon de sang. Il a finalement été observé ≥ 18 % d'hémolyse (engendrée par l'utilisation du protocole recommandé dans la directive CLSI EP07-A2, annexe G, G1 – Procédure de choc osmotique) provoquant des interférences. C'est pourquoi les échantillons hémolysés devrait être écartés. Pour la détermination des CD4 et CD4% dans les échantillons de sang total avec des dosages CyFlow™ Counter, aucun autre anticoagulant que l'EDTA ne doit être utilisé. Pour toutes questions supplémentaires concernant la performance du produit, veuillez contacter votre représentant Sysmex local.

17 Bibliographie

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18 Coordonnées du fabricant

Fabricant
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Allemagne
www.sysmex-partec.com
Tél +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

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La fiche de données de sécurité de ce produit est disponible à l'adresse suivante : www.sysmex-partec.com/services.
Cy et CyDye sont des marques déposées de GE Healthcare.

sysmex

Instrucciones de uso

Identificación del reactivo para DIV
Nombre del producto:
Kit CD4% easy count
REF 05-8405

Contenido::

Vial con CD4 mAb PE	REF 05-8405-01
Vial con CD45 mAb PE-Cy5	REF 05-8405-02
Botella con solución amortiguadora 1	REF 05-8405-03
Botella con solución amortiguadora 2	REF 05-8405-04

1 Descripción

Especificidad	CD4 humano	CD45 humano
Isotipo	IgG1	IgG1
Contenido	100 tests	100 tests
Fluorocromo	PE	PE-Cy5
λ de excitación (nm)	532 / 488	532 / 488
Emisión máxima (nm)	578	670

2 Uso previsto
IVD Para diagnóstico in vitro.

El kit CD4% easy count es un test cuantitativo manual de DIV de cuatro componentes para el marcaje de leucocitos y una subpoblación de linfocitos en sangre entera venosa adulta con EDTA, que pueden a continuación enumerarse con el citómetro de flujo para DIV CyFlow™ Counter de Sysmex Partec. La concentración de linfocitos T CD4 y el CD4% de linfocitos da las muestras de sangre son indicadores útil para iniciar o supervisar el tratamiento de personas inectadas por el VIH junto con otros hallazgos clínicos y de laboratorio.

El test está previsto para su uso por parte de profesionales sanitarios con la formación correspondiente.

3 Principio del método de análisis
Se mezcla una cantidad equivalente de una muestra de sangre entera con EDTA con dos anticuerpos (CD4 y CD45), cada uno conjugado con un fluorocromo diferente para marcar poblaciones celulares específicas. Tras un tiempo fijo de incubación, se añaden las dos soluciones amortiguadoras y la muestra está lista para su análisis en el citómetro de flujo CyFlow™ Counter. La fuente de luz excita el tinte fluorescente vinculado con la célula teñida y la luz emitida se detecta mientras un volumen preciso de muestra de sangre pasa por el instrumento. La concentración de poblaciones celulares específicas se calcula con el software integrado. Para más información, consulte las instrucciones de uso de CyFlow™ Counter (CY-S-3022IFUEN).

4 Almacenamiento y tiempo de conservación tras abrir por primera vez
1. Almacenamiento:
Almacene los reactivos de anticuerpos y solución amortiguadora en un lugar oscuro a 2 - 8 °C. No congele ni exponga los reactivos a temperaturas elevadas o a la luz solar directa.
2. Tiempo de conservación tras abrir por primera vez:
Cierre siempre la botella después de su uso y utilice puntas de pipeta nuevas cada vez que tome muestras del reactivo para evitar la contaminación.
En estas condiciones de almacenamiento, el kit CD4% easy count permanecerá estable hasta la fecha de caducidad impresa en la etiqueta del kit.

5 Componentes
mAb CD4-PE y mAb CD45-PE-Cy5 son anticuerpos monoclonales murinos suministrados en una solución amortiguadora PBS con un 0,2% de SAB y un 0,09% de azida de sodio. Las soluciones amortiguadoras 1 y 2 son soluciones con base de PBS que contienen un 0,09% de azida de sodio.

6 Indicios de deterioro
El reactivo de anticuerpos y la solución amortiguadora son líquidos transparentes. No utilice el reactivo si aparece cualquier tipo de turbidez o contaminación. Si tiene alguna pregunta con respecto al funcionamiento o la calidad del producto, contacte con su representante local de Sysmex y solicite asistencia técnica.

7 Precaución y advertencias
Los reactivos contienen 14 mM de azida de sodio como conservante. Dada su reducida concentración de azida de sodio, no requiere un etiquetado como producto de riesgo, pero se deben observar las precauciones de seguridad normales para la manipulación de sustancias químicas. Consulte la ficha técnica de seguridad para obtener una lista completa de advertencias y precauciones.

8 Equipamiento adicional necesario

Instrumental necesario:	CyFlow™ Counter (REF No.: CY-S-3022)
Equipamiento requerido:	Tubos de ensayo de 3.5 ml (REF No.: 04-2000) Una pipeta verificada de 10 µl y puntas de pipeta Una pipeta verificada de 20 µl y puntas de pipeta Una pipeta verificada de 100 – 1000 µl y puntas de pipeta Sistema de extracción de sangre venosa con EDTA como anticoagulante Cronómetro

9 Preparación del reactivo
CD4 mAb PE (REF No.: 05-8405-01), el reactivo de anticuerpos está listo para su uso.
CD45 mAb PE-Cy5 (REF No.: 05-8405-02), el reactivo de anticuerpos está listo para su uso.
Solución amortiguadora 1 (REF No.: 05-8405-03), la solución amortiguadora está lista para su uso.
Solución amortiguadora 2 (REF No.: 05-8405-04), la solución amortiguadora está lista para su uso.

10 Principios básicos de extracción, manipulación y almacenamiento de muestras

ADVERTENCIA ⚠ *Todas las muestras y materiales biológicos se consideran material de riesgo biológico y deberían manipularse como si fueran potencialmente infecciosos. Se deben observar las precauciones de seguridad y procedimientos de manipulación adecuados conforme a la legislación y normativa.*

- La muestra de sangre debe ser una muestra de sangre entera venosa, extraída con un sistema de extracción de sangre que contenga EDTA como anticoagulante.
- La muestra de sangre debe transportarse en un contenedor oscuro, protegido de la luz y no expuesto a temperaturas elevadas. Un contenedor de transporte adecuado es, por ejemplo, una caja de poliestireno extruido con material refrigerante. Las mejores condiciones de transporte serían inferiores a 25 °C si el transporte no durará más de 6 horas después de la donación.
- Para el transporte y el tiempo de almacenamiento de más de 6 horas, la sangre debe mantenerse a 2 - 8 °C.
- No se puede congelar y descongelar la muestra de sangre.
- En condiciones óptimas la muestra de sangre debería ser reciente, es decir, no deberían haber pasado más de seis horas entre la extracción y el análisis de la muestra.
- Las muestras de sangre se pueden utilizar hasta 24 horas después de la extracción si se almacenan en el frigorífico a 2 - 8 °C.
- Los tubos con las muestras de sangre se deben voltear con suavidad de 8 a 10 veces antes de ser utilizados.
- No utilice muestras de sangre coagulada.

11 Procedimiento de análisis

AVISO *El pipeteado inverso es fundamental para la precisión, en particular cuando se dispensan volúmenes de muestra viscosos y muy pequeños. Para el pipeteado de sangre completa, recomendamos emplear una pipeta electrónica calibrada que esté preprogramada para funcionar en modo de pipeteado inverso.*
Si no hay disponible una pipeta electrónica, siga las presentes instrucciones para llevar a cabo un pipeteado inverso manual:
- *Presione el botón de funcionamiento hasta el segundo tope. Deje que el botón de funcionamiento ascienda por completo; la muestra sobrante se aspirará a la punta.*
- *Presione el botón de funcionamiento hasta el primer tope para expulsar un volumen concreto de sangre, dejando así la muestra sobrante dentro de la punta.*
- *Elimine la punta de la pipeta que contiene la muestra de sangre sobrante en un contenedor para residuos médicos.*

Tenga en cuenta que no debe utilizar el pipeteado inverso para soluciones de anticuerpos ni soluciones tampón.

11.1 Tinción
- Voltee con suavidad de 8 a 10 veces el tubo de extracción de sangre con EDTA que contiene la muestra de sangre.
- Pase con la pipeta 20 µl de la muestra de sangre entera con EDTA al fondo de un tubo de muestra y evite manchar la pared interior del tubo con la sangre de la punta de la pipeta. Deseche la punta de la pipeta.
- Añada 10 µl de mAb CD4-PE directamente en la muestra de sangre y mézclelo suavemente, evitando manchar la pared interior del tubo con la sangre de la punta de la pipeta. Deseche la punta de la pipeta.
- Añada 10 µl de mAb CD45-PE-Cy5 directamente en la muestra de sangre y mézclelo suavemente, evitando manchar la pared interior del tubo con la sangre de la punta de la pipeta. Deseche la punta de la pipeta.
- Incube la mezcla durante 15 minutos a 15 - 30 °C en la oscuridad.
- Añada 400 µl de la solución amortiguadora 1 y agite brevemente con la agitadora vorticial, o bien golpee suavemente el tubo para mezclar la muestra. Deseche la punta de la pipeta.
- Añada 400 µl de la solución amortiguadora 2 y agite brevemente con la agitadora vorticial, o bien golpee suavemente el tubo para mezclar la muestra. Deseche la punta de la pipeta.
- Agite brevemente mediante agitadora vorticial, o bien golpee suavemente el tubo antes del análisis con el CyFlow™ Counter.

AVISO *Tras añadir la solución amortiguadora 1, las muestras pueden almacenarse hasta 2 horas a 2 - 8 °C a oscuras. No utilice la muestra asado este periodo o si no se ha preparado correctamente. Las muestras se deben analizar dentro de un periodo máximo de 10 minutos tras la adición de la solución amortiguadora 2.*

11.2 Análisis de la muestra
En las instrucciones de uso de CyFlow™ Counter (CY-S-3022IFUEN) puede consultar el modo del empleo del equipo antes de analizar la muestra. Los procedimientos de puesta en marcha y el proceso de control de calidad interno deben completarse con éxito antes de iniciar el análisis de la muestra.
- Elija y cargue la configuración para la medición de CD4% desde la barra de menú de CyFlow™ Counter.
- Inserte el tubo de muestras con la muestra de sangre preparada en el acoplamiento para muestras.
- Inicie la medición.
- Tras el análisis de la muestra, el dispositivo se detiene y se limpia automáticamente.
- Retire y deseche la muestra de sangre conforme a los procedimientos locales de seguridad en laboratorio frente a riesgos biológicos.

No inicie una segunda medición con el mismo tubo de muestras. Si se requiere una repetición de la medición, se debe preparar un segundo tubo de muestras conforme a 11.1.

11.3 Adquisición y análisis de datos
El análisis de datos con CyFlow™ Counter solo es posible si el control de calidad interno del instrumento ha resultado satisfactorio. Consulte las instrucciones de uso del CyFlow™ Counter (CY-S-3022IFUEN) para realizar, si fuera necesario, el procedimiento de inicio y mantenimiento del dispositivo. Los resultados de contar el número de células CD4-T, el porcentaje de células CD4 y el recuento de linfocitos se presentarán en la pantalla. Si las agrupaciones de células teñidas no se pueden diferenciar claramente de las señales de ruido de fondo en el diagrama de dispersión lateral de CD45 (véase la figura 2), o bien no se pueden diferenciar claramente las agrupaciones de células positivas para CD4, las células negativas para CD4 y los monocitos en el diagrama de dispersión lateral de CD4, entonces habrá que repetir la preparación de la muestra y la medición.

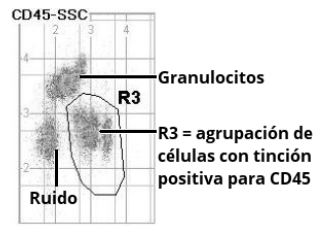

Figura 1: Ejemplo de agrupaciones bien diferenciadas de granulocitos, otros leucocitos con tinción positiva para CD45 y señales de ruido.

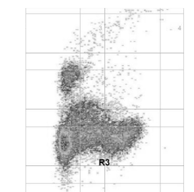

Figura 2: Ejemplo de mala separación entre ruido de fondo y los leucocitos teñidos con CD45 positivo. Esta muestra no es analizable.

Los resultados serán incorrectos si el posicionamiento de la puerta de las células teñidas con CD45 positivo (R3) no se realiza con precisión.

12 Cálculo de los resultados del análisis
El cálculo de la concentración celular forma parte del análisis de datos informático, el cual suministra el resultado en CD4%, células CD4+ por µl de muestra de sangre y linfocitos por µl de muestra de sangre después de cada medición.
Para más información, consulte las instrucciones de uso de CyFlow™ Counter (CY-S-3022IFUEN).

13 Interpretación de los resultados
El virus de la inmunodeficiencia humana (VIH) es uno de los principales motivos del agotamiento de las células CD4, que debilita el sistema inmunitario. Los resultados deben ser interpretados por los médicos, en conjunción con las pautas de tratamiento del VIH aplicables localmente.

14 Procedimiento de control
Se debe realizar un control de calidad apropiado conforme a las normativas locales y nacionales. Se han comercializado diversas muestras de sangre estabilizada, pero solo unas pocas son adecuadas cuando se utilizan determinados citómetros de flujo. Si no se cumple el criterio de aceptación del material de control específico, no prosiga con el análisis de la muestra del paciente y póngase en contacto con su representante local de Sysmex.
Para más información sobre el uso de material de control adecuado, póngase en contacto con su representante local de Sysmex.

15 Eficacia diagnóstica
15.1 Especificidad
El anticuerpo MEM-241 reconoce el antígeno CD4, una glucoproteína transmembranaria de 55 kDa expresada en un subconjunto de linfocitos T (células T cooperadoras) y también en monocitos, macrófagos y granulocitos. Congreso de HCDM (anteriormente HLDA VIII), mayo de 2006, Quebec, Canadá. Código WS: M241.
El anticuerpo MEM-28 reacciona con todas las formas alternativas de antígeno humano CD45 (antígeno leucocitario común), una proteína transmembranaria de tipo I monocatenaria de 180-220 kDa con elevada concentración en todas las células de origen hematopoyético, excepto los eritrocitos y las plaquetas. HLDA III. Código WS: NL 833a.

15.2 Capacidad de detección
La evaluación del límite de inclusión (LI), el límite de detección (LD) y el límite de cuantificación (LC) se realizó de acuerdo con las indicaciones de la directriz CLSI EP17-A2.
LI: ≤50% del LD
LD: ≤20 células CD4/µl
LC: ≤40 células CD4/µl

15.3 Repetibilidad
Variación intra e interanalítica:
Los valores de coeficiente de variación (CV) suponen menos del 10% para valores de CD4 ≥ 200 células T CD4/µl.
Los valores de coeficiente de variación (CV) suponen menos al 10% para valores de CD4% ≥ 25%.
Los valores de coeficiente de variación (CV) suponen menos del 15% para valores absolutos de CD4 inferiores a 200 células T CD4/µl.
Los valores de desviación estándar (SD) ≤ 2,5% para valores de CD4% inferiores al 25%.

15.4 Linealidad
El intervalo de medición del ensayo y la linealidad de la medición son válidos en el intervalo:
CD4 absoluto: 40-2500 células T CD4/µl
CD4%: 4 – 60%

15.5 Concordancia
Las muestras de sangre entera con EDTA se tiñeron con el kit CD4% easy count y se analizaron en el CyFlow™ Counter (CyView™ 2.11). Se compararon los valores de CD4 absoluto y CD4% con los resultados de un BD FACSCalibur utilizando BD Tritest CD3/CD4/CD45 y tubos BD Trucount y se puso de manifiesto una divergencia del -22,56% (CD4 absoluto) y del +2,1% (CD4%) entre ambos métodos.

16 Limitaciones

CUIDADO ⚠ *El uso de los kits de Sysmex Partec CD4% easy count (05-8405) no está validado para pacientes pediátricos y adolescentes.*

CUIDADO ⚠ *Las mediciones de los pacientes de CD4 y CD4% realizadas con los ensayos de Sysmex Partec no deben utilizarse junto con las medidas obtenidas de otros métodos del fabricante para determinar CD4 y CD4%. Use los ensayos solo con los instrumentos de Sysmex Partec. Los valores obtenidos usando otros equipos o ensayos no son intercambiables.*

Se analizaron diferentes sustancias endógenas y exógenas conforme a la directriz CLSI EP07-A2 para detectar posibles efectos de interferencia que pudieran influir en el análisis de la muestra de sangre. Finalmente, se observó un ≥18% de hemólisis (provocada por el uso del protocolo recomendado en CLSI EP07-A2, apéndice G, G1, «Procedimiento de choque osmótico») como muestra de un efecto de interferencia. Por ese motivo, se deben rechazar las muestras hemolizadas. Para la determinación de CD4 y CD4% en muestras de sangre total con los ensayos CyFlow™ Counter, no se debe utilizar ningún otro anticoagulante que el EDTA.
Si tiene alguna pregunta más con respecto al funcionamiento del producto, póngase en contacto con su representante local de Sysmex.

17 Referencias bibliográficas

1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
2) L. Zijenah, G. Kadzirange, S. Madzime, M. Borok, C. Mudiwa, O. Tobaiwa, M. Mucheche, S. Rusakaniko, D. Katzenstein. (2006). Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-I infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. Journal of Translational Medicine 4:33
3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
4) S. Kohreanudom, S. Dettraira. (2012). Evaluation of the Partec Single-Platform Volumetric CyFlow® Counter System for Determining Percentage and Absolute Numbers of CD4 T Lymphocytes in HIV/AIDS Thai Patients. Thai AIDS J, 25:1-10
5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Contacto del fabricante

Fabricante
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Alemanha
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Número de versión de las instrucciones de uso y fecha de publicación

Revisión: Rev-07_10-09-2020_CN 1563
Publicada por: Sysmex Partec GmbH

Puede consultar la ficha técnica de seguridad de este producto en www.sysmex-partec.com/services.

Instruções de utilização

Identificação do reagente IVD
Nome do produto:
CD4% easy count kit
REF 05-8405

Conteúdo:

Recipiente contendo CD4 mAb PE	REF 05-8405-01
Recipiente contendo CD45 mAb PE-Cy5	REF 05-8405-02
Frasco contendo Tampão 1	REF 05-8405-03
Frasco contendo Tampão 2	REF 05-8405-04

1 Especificação

Especificidade	CD4 humano	CD45 humano
Isótipo	IgG1	IgG1
Conteúdo	100 testes	100 testes
Fluorocromo	PE	PE-Cy5
λ excitação (nm)	532 / 488	532 / 488
Máximo de emissão (nm)	578	670

2 Uso previsto
IVD Para uso no diagnóstico in vitro.

O CD4% easy count kit é um teste IVD manual quantitativo com quatro componentes, para a marcação de leucócitos e de uma subpopulação de linfócitos em sangue total EDTA venoso de adulto, que podem ser posteriormente enumerados no citómetro de fluxo IVD Sysmex Partec CyFlow™ Counter.
A concentração de células CD4 T e CD4% de linfócitos em amostras de sangue são indicadores útil para o iníciação ou o acompanhamento do tratamento para pacientes vom VIH positivo em conjugação com outros resultados laboratoriais e clínicos.
O teste destina-se a ser realizado por profissionais de saúde devidamente qualificados.

3 Princípio do método de análise
Uma alíquota de uma amostra de sangue total EDTA é misturada com dois anticorpos (CD4 e CD45), cada um deles conjugado com um fluorocromo diferente para marcar populações de células específicas. Após um tempo de incubação fixo, as duas soluções tampão são adicionadas e a amostra fica pronta para análise no citómetro de fluxo CyFlow™ Counter.
A fonte de luz excita o corante fluorescente ligado à célula corada e a luz emitida é detectada enquanto um volume preciso de amostra de sangue passa pelo instrumento. A concentração das populações de células específicas é calculada pelo software integrado.
Para mais informações, consulte as instruções de utilização do CyFlow™ Counter (CY-S-3022IFUEN).

4 Armazenamento e prazo de validade após a primeira abertura
1. Armazenamento:
Armazene os reagentes tampão e de anticorpos entre 2 - 8 °C no escuro. Não congele nem exponha os reagentes a temperaturas elevadas e mantenha-os protegidos da luz solar direta.
2. Prazo de validade após a primeira abertura:
Feche sempre o frasco após a utilização e utilize pontas de pipeta novas de cada vez que o reagente é amostrado, para evitar a contaminação.
Sob estas condições de armazenamento, o CD4% easy count kit irá permanecer estável até à data de validade impressa no rótulo do kit.

5 Componentes
O CD4 mAb PE e o CD45 mAb PE-Cy5 são anticorpos monoclonais murinos fornecidos em tampão PBS com 0,2% de BSA e 0,09% de azida de sódio. Tampão 1 e tampão 2 são soluções baseadas em PBS contendo 0,09% de azida de sódio.

6 Indício de deterioração
As soluções tampão e de anticorpos são líquidos transparentes. Não utilize os reagentes caso surja qualquer tipo de turbidez ou contaminação.
Para questões relativas ao desempenho ou qualidade do produto, entre em contacto com o representante local da Sysmex para obter suporte técnico.

7 Precauções e advertências
Os reagentes contêm 14 mM de azida de sódio como conservante. A baixa concentração de azida de sódio não exige a rotulagem como substância perigosa, mas é necessário respeitar as precauções de segurança normais relativas ao manuseamento dos produtos químicos. Consulte a ficha de segurança para obter uma lista completa das advertências e precauções.

8 Additional required equipment

Instrumento necessário:	CyFlow™ Counter (REF No.: CY-S-3022)
Equipamento necessário:	Tubos de amostra de 3.5 ml (REF No.: 04-2000) Uma pipeta verificada de 10 µl e pontas de pipeta Uma pipeta verificada de 20 µl e pontas de pipeta Uma pipeta verificada de 100 - 1000 µl e pontas de pipeta Sistema de colheita de sangue venoso com EDTA como anticoagulante Cronómetro

9 Preparação do reagente
CD4 mAb PE (REF No.: 05-8405-01), O reagente de anticorpos está pronto a usar.
CD45 mAb PE-Cy5 (REF No.: 05-8405-02), O reagente de anticorpos está pronto a usar.
Tampão 1 (REF No.: 05-8405-03), A solução tampão está pronta a usar.
Tampão 2 (REF No.: 05-8405-04), A solução tampão está pronta a usar.

10 Colheita, manuseamento e armazenamento da amostra principal

ADVERTÊNCIA ⚠ *Todas as amostras biológicas e materiais são considerados como perigosos e devem ser manuseados como sendo capazes de transmitir infeções. É necessário aplicar precauções de segurança e procedimentos de manuseamento adequados em conformidade com as leis e regulamentos aplicáveis.*

- A amostra de sangue tem de ser uma amostra de sangue total venoso, recolhida com um sistema de colheita de sangue, que contém EDTA como anticoagulante.
- A amostra de sangue tem de ser transportada num recipiente escuro, protegida da luz e não exposta a temperaturas elevadas. Um recipiente de transporte adequado é, p. ex., uma caixa de espuma de poliestireno com material de refrigeração. As melhores condições de transporte seriam inferiores a 25 °C se o transporte não durar mais de 6 horas após a doação.
- Para transporte e tempo de armazenamento superior a 6 horas, o sangue deve ser mantido a 2 - 8 °C.
- Não congele e descongele a amostra de sangue.
- A fim de assegurar as melhores condições, a amostra de sangue deve ser fresca, ou seja, o tempo de colheita e análise da mesma não pode ser superior a seis horas.
- As amostras de sangue podem ser utilizadas até 24 horas após a colheita, desde que tenham sido armazenadas no frigorífico entre 2 - 8 °C.
- Antes da utilização, os tubos de amostra que contêm o sangue devem ser invertidos cuidadosamente 8 - 10 vezes.
- Não utilize amostras de sangue coagulado

11 Procedimento de análise

NOTA *A pipetagem reversa é fundamental para a precisão, sobretudo ao dispensar volumes de amostra viscosos e muito reduzidos. Para a pipetagem de sangue total, recomendamos a utilização de uma pipeta eletrónica calibrada pré-programada para operar em modo de pipetagem reversa.*
Se não estiver disponível uma pipeta eletrónica, seguir estas instruções para a pipetagem reversa manual:
- *Premir o botão de operação até ao segundo ponto de retenção. Deixar o botão de operação subir completamente; o excesso da amostra é aspirado para a ponta.*
- *Premir o botão de operação até ao primeiro ponto de retenção para expulsar um volume exato de sangue, deixando o excesso da amostra na ponta.*
- *Deitar a ponta da pipeta com a amostra de sangue em excesso no contentor de lixo hospitalar.*

Ter em atenção que a pipetagem reversa não pode ser usada para anticorpos ou soluções-tampão.

11.1 Coloração
- Inverta cuidadosamente a amostra de sangue no tubo de colheita de sangue EDTA 8 - 10 vezes.
- Pipete 20 µl de amostra de sangue total EDTA até ao fundo de um tubo de amostra, evitando deixar um rasto de sangue na parede interior do tubo com a ponta de pipeta. Descarte a ponta da pipeta.
- Adicione 10 µl de CD4 mAb PE diretamente na amostra de sangue e misture cuidadosamente, evitando deixar um rasto de sangue na parede interior do tubo com a ponta de pipeta. Descarte a ponta da pipeta.
- Adicione 10 µl de CD45 mAb PE-Cy5 diretamente na amostra de sangue e misture cuidadosamente, evitando deixar um rasto de sangue na parede interior do tubo com a ponta de pipeta. Descarte a ponta da pipeta.
- Incube a mistura durante 15 minutos noescuro à 15 - 30 °C.
- Adicione 400 µl de tampão 1 e centrifugue brevemente ou bata cuidadosamente no tubo para misturar a amostra. Descarte a ponta da pipeta.
- Adicione 400 µl de tampão 2 e centrifugue brevemente ou bata cuidadosamente no tubo para misturar a amostra. Descarte a ponta da pipeta.
- Centrifugue brevemente ou bata cuidadosamente no tubo antes de efetuar a análise com o CyFlow™ Counter.

NOTA *Após a adição do tampão 1, as amostras podem ser armazenadas num ambiente escuro e a 2 - 8 °C durante um período máximo de 2 horas. Não use a amostra depois desse período ou caso esta não tenha sido devidamente preparada.*
Após a adição do tampão 2, as amostras têm de ser analisadas dentro de 10 minutos.

11.2 Análise da amostra
Consulte as instruções de utilização do CyFlow™ Counter para obter informações sobre como operar o instrumento antes de analisar a amostra. Os procedimentos de arranque e o procedimento interno de controlo da qualidade têm de ser concluídos com êxito antes da análise da amostra.
- Escolha e carregue a configuração para medição de CD4% na barra de menu do CyFlow™ Counter.
- Insira o tubo da amostra com a amostra de sangue preparada na porta de amostragem.
- Inicie a medição.
- Após o processamento da amostra, o instrumento para e limpa-se automaticamente.
- Retire e elimine a amostra de sangue de acordo com os procedimentos de segurança locais do laboratório em termos de perigo biológico.

Não inicie uma segunda medição com o mesmo tubo de amostra. Se for necessária uma duplicação ou repetição da medição, terá de ser preparado um segundo tubo de amostra tal como descrito em 11.1.

11.3 Aquisição e análise de dados
A análise de dados com o CyFlow™ Counter só é possível caso o controlo de qualidade interno do instrumento tenha sido executado com êxito.
Consulte as instruções de utilização do CyFlow™ Counter (CY-S-3022IFUEN) para informações sobre o procedimento de arranque e a manutenção do dispositivo, se necessário.
Os resultados da contagem do número de células CD4-T, determinando a porcentagem de células CD4 e a contagem de linfócitos serão apresentados na tela. Se os aglomerados das células coradas contra os sinais de ruído de fundo no gráfico de pontos CD45 – SSC não forem claramente separáveis (ver figura 2), ou se os aglomerados de células positivas CD4, células negativas CD4 e monócitos no gráfico de pontos CD4 – SSC não forem claramente separáveis, a preparação e medição da amostra têm de ser repetidas..

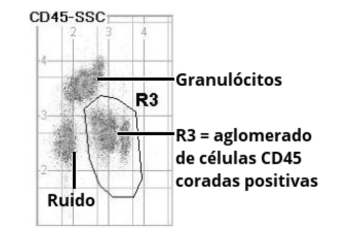

Figura 1: Exemplo de aglomerados de granulócitos bem separados, outros leucócitos corados positivos CD45 e sinais de ruído.

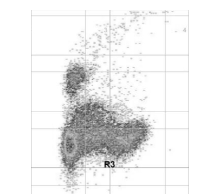

Figura 2: Exemplo de má separação entre ruído e leucócitos corados positivos CD45. Esta amostra não é analisável.

Os resultados serão incorretos se o posicionamento da porta de células CD45 positivas (R3) não for feito com precisão.

12 Cálculo dos resultados da análise
O cálculo da concentração de células faz parte da análise de dados baseada em software, que fornece o resultado em células CD4% e CD4+ por µl de amostra de sangue e linfócitos por µl de amostra de sangue, após cada medição.
Para mais informações, consulte as instruções de utilização do CyFlow™ Counter (CY-S-3022IFUEN).

13 Interpretação dos resultados
O vírus da imunodeficiência humana (VIH) é uma das principais razões para a depleção de células CD4, que debilita o sistema imunológico. Os resultados devem ser interpretados pelos médicos, em conjunto com as diretrizes de tratamento do VIH aplicáveis localmente.

14 Procedimento de controlo
Deve ser realizado um controlo da qualidade apropriado de acordo com regulamentos locais e nacionais.
Encontra-se disponível no mercado uma variedade de amostras de sangue estabilizado, mas poucas são adequadas para a utilização de certos citómetros de fluxo.
Se não for cumprido o critério de aceitação do material de controlo individual, não avance para o teste da amostra do paciente e entre em contacto com o seu representante local da Sysmex.
Para mais informações acerca do uso de material de controlo adequado, entre em contacto com o seu representante local da Sysmex.

15 Características de desempenho
15.1 Especificidade
O anticorpo MEM-241 reconhece o antigénio CD4, uma glicoproteína transmembrana de 55 kDa expressa num subconjunto de linfócitos T (células T „auxiliares") e também em monócitos, granulócitos e macrófagos do tecido. Conferência sobre HCDM (o anterior HLDA VIII), maio de 2006, Quebeque, Canadá; código WS M241
O anticorpo MEM-28 reage com todas as formas alternativas de antigénio CD45 humano (antigénio leucocitário comum), uma proteína transmembrana tipo I de cadeia simples de 180-220 kDa, expressa a nível superior em todas as células de origem hematopoiética, exceto nos eritrócitos e plaquetas. HLDA III; código WS NL 833a

15.2 Capacidade de deteção
A avaliação para Limite de valores em branco (LoB), Limite de deteção (LoD) e Limite de quantificação (LoQ) foi realizada de acordo com as especificações da diretriz CLSI EP17-A2.
LoB: ≤50% of LoD
LoD: ≤20 CD4 células/µl
LoQ: ≤40 CD4 células/µl

15.3 Repetibilidade
Variação intra e entre ensaios:
Os valores CV são inferiores a 10% para valores CD4 de 200 células T CD4/µl ou superiores.
Os valores CV são inferiores a 10% para valores CD4% de 25% ou superiores.
Os valores CV são inferiores a 15% para valores absolutos de CD4 inferiores a 200 células T CD4/µl.
Os valores do desvio padrão (SD) ≤ 2,5% para valores de CD4% são inferiores a 25%.

15.4 Linearidade
A gama de medição do ensaio e linearidade da medição são válidas ao longo da gama:
CD4 absoluto:: 40-2500 CD4 T-células/µl
CD4%: 4 – 60%

15.5 Veracidade
Uma amostra de sangue total EDTA foi corada com o CD4% easy count kit e analisada no CyFlow™ Counter (CyView™ 2.11). Os valores de CD4 absoluto e CD4% foram comparados com os resultados de um BD FACSCalibur usando BD Tritest CD3/CD4/CD45 e tubos BD Trucount, tendo-se revelado um desvio de -22,56% (CD4 absoluto) e +2,1% (CD4%) entre ambos os métodos.

16 Limitações

CUIDADO ⚠ *Os kits Sysmex Partec CD4% easy count (05-8405) não foram validados para utilização com pacientes pediátricos e adolescentes.*

CUIDADO ⚠ *As medições de paciente de CD4 e de CD4% feitas com os ensaios Sysmex Partec não devem ser usadas de forma independente com outros métodos do fabricante para determinar CD4 e CD4%.*
Use os ensaios apenas com os instrumentos Sysmex Partec. Os valores obtidos usando outros equipamentos ou ensaios podem não ser intercambiáveis.

Foram testadas diversas substâncias endógenas e exógenas relativamente à diretriz CLSI EP07-A2 para identificar possíveis efeitos de interferência que possam exercer influência sobre a análise da amostra de sangue.
Finalmente, observou-se ≥ 18% de hemólise (induzida usando o protocolo recomendado na CLSI EP07-A2, Apêndice G, G1 - Procedimento de Choque Osmótico) para demonstrar um efeito de interferência. Por esse motivo, deverão ser rejeitadas amostras hemolisadas. Para a determinação de CD4 e CD4% em amostras de sangue total com ensaios CyFlow™ Counter, nenhum outro anticoagulante que o EDTA deve ser usado.
Para outras questões relativas ao desempenho do produto, entre em contacto com o seu representante local da Sysmex.

17 Referências bibliográficas

1) M. Fryland, P. Chaillet, R. Zachariah, A. Barnaba, L. Bonte, R. Andereassen, S. Charrondière, R. Teck, O. Didakus. (2006). The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(10):980-5
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3) H. Karcher, D. Bohning, R. Downing, S. Mashate, G. Harms. (2006). Comparison of two alternative methods for CD4+ T-cell determination (Coulter manual CD4 count and CyFlow) against standard dual platform flow cytometry in Uganda. Clinical Cytometry, 70B (3):163-69
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5) D. Wade, P.A. Diaw, G. Daneau, M. Camara, T.N. Dieye, S. Mboup, L. Kestens. (2013). CD4 T-Cell Enumeration in a Field Setting: Evaluation of CyFlow Counter Using the CD4 Easy Count Kit-Dry and Pima CD4 Systems. PLoS ONE, 8(9): e75484

18 Contacto do fabricante

Fabricante
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Alemanha
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Número de versão das instruções de utilização e data da pu blicação

Revisão: Rev-07_10-09-2020_CN 1563
Publicado por: Sysmex Partec GmbH

A ficha de segurança deste produto está disponível em www.sysmex-partec.com/services.

Инструкция по эксплуатации

IVD CE

Идентификация реагента для диагностики in vitro (IVD)
Наименование продукта
CD4% easy count kit
REF 05-8405

Содержимое:

Ампула, содержащая CD4 mAb PE	REF 05-8405-01
Ампула, содержащая CD45 mAb PE-Cy5	REF 05-8405-02
Флакон, содержащий буфер 1	REF 05-8405-03
Флакон, содержащий буфер 2	REF 05-8405-04

1 Спецификация

Специфичность	CD4 человека	CD45 человека
Изотип	IgG1	IgG1
Содержимое	100 тестов	100 тестов
Флуорохром	PE	PE-Cy5
λ возбуждение (нм)	532 / 488	532 / 488
Максимальная эмиссия (нм)	578	670

2 Предполагаемое использование
IVD Для использования в диагностике in vitro.

Набор CD4% easy count kit — это четырехкомпонентный набор, предназначенный для ручного количественного анализа in vitro, используемый для мечения лейкоцитов и субпопуляций лимфоцитов в цельной венозной крови с ЭДТА взрослых пациентов и их последующего подсчета с помощью проточного цитометра для диагностики in vitro CyFlow™ Counter компании Sysmex Partec.
Концентрация CD4 Т-клеток и CD4% лимфоцитов в образцах крови являются полезными показателями для начала или последующего лечения пациентов с ВИЧ-инфекцией в сочетании с другими лабораторными и клиническими данными.
Анализ должен проводиться обученными специалистами здравоохранения.

3 Принцип метода подсчета
Аликвоту образца цельной крови с ЭДТА смешивают с двумя типами антител (CD4 и CD45), конъюгированных с разными флуорохромами для мечения клеток определенных популяций. После инкубирования в течение фиксированного времени добавляют два буферных раствора, после чего образец готов для анализа на проточном цитометре CyFlow™ Counter.
Источник света возбуждает флуоресцентный краситель, связанный с окрашенной клеткой, и излучаемый свет обнаруживается, когда точный объем образца крови проходит через инструмент.
Концентрация клеток определенных популяций рассчитывается с помощью интегрированного программного обеспечения.
Для получения дополнительной информации см. ИЭ цитометра CyFlow™ Counter (CY-S-3022IFUEN).

4 Хранение и срок годности после первого вскрытия
1. Хранение:
Антитела и буферные реагенты следует хранить при температуре 2 - 8 °C в темном месте. Реагенты нельзя замораживать, подвергать воздействию высоких температур и прямых солнечных лучей.
2. Срок годности после первого вскрытия:
Следует всегда закрывать флакон после использования и каждый раз при взятии реагента пользоваться новым наконечником для пипетки с целью предотвращения контаминации.
При таких условиях хранения реагенты набора CD4% easy count kit будут стабильны до окончания срока годности, указанного на его этикетке.

5 Компоненты
CD4 mAb PE и CD45 mAb PE-Cy5 представляют собой моноклональные мышиные антитела в фосфатно-солевом буфере (PBS), содержащем 0,2 % БСА и 0,09 % азида натрия. Буфер 1 и буфер 2 представляют собой растворы на основе PBS, содержащие 0,09 % азида натрия.

6 Признаки ухудшения качества
Антитела и буферные растворы представляют собой прозрачную жидкость. Не следует использовать реагенты при появлении любого рода помутнения или признаков контаминации.
При наличии вопросов о характеристиках или качестве продукта обращайтесь в службу технической поддержки местного представительства компании Sysmex.

7 Предупреждения и меры предосторожности
Реагенты содержат 14 мМ азида натрия в качестве консерванта. Низкая концентрация азид натрия не требует маркировки опасности, однако при их использовании следует соблюдать стандартные меры предосторожности по безопасному обращению с химическими веществами. Полный перечень предупреждений и мер предосторожности приведен в паспорте безопасности (ПБ) продукта.

8 Дополнительное требуемое оборудование

Требуемый прибор:	CyFlow™ Counter (REF No.: CY-S-3022)
Требуемое оборудование:	пробирки объемом 3,5 мл (REF No.: 04-2000) поверенная пипетка объемом 10 мкл и наконечники для пипетки поверенная пипетка объемом 20 мкл и наконечники для пипетки поверенная пипетка объемом 100 - 1000 мкл и наконечники для пипетки система для сбора венозной крови с ЭДТА в качестве антикоагулянта секундомер

9 Приготовление реагента
CD4 mAb PE (REF No.: 05-8405-01), Реагент с антителами готов к использованию.
CD45 mAb PE-Cy5 (REF No.: 05-8405-02), Реагент с антителами готов к использованию.
буфер 1 (REF No.: 05-8405-03), Буферный раствор готов к использованию.
буфер 2 (REF No.: 05-8405-04), Буферный раствор готов к использованию.

10 Взятие основного образца, обращение с ним и его хранение

ПРЕДУПРЕЖДЕНИЕ ⚠ *Все биологические образцы и материалы считаются биологически опасными, с ними следует обращаться как с потенциальным источником инфекции. При обращении с ними следует соблюдать надлежащие меры предосторожности и требования безопасности в соответствии с применимыми законами и нормативами*

- Образцом должен служить образец цельной венозной крови, взятый с помощью системы для сбора крови, содержащей ЭДТА в качестве антикоагулянта.
- Образец крови следует транспортировать в непрозрачном контейнере, защищать от воздействия света и не подвергать воздействию высоких температур. Подходящим транспортным контейнером является, например, пенопластовый (Styrofoam) контейнер с охлаждающим материалом. Лучшие транспортные условия будут ниже 25 °C, если транспорт не займет больше 6 часов после пожертвования.
- Для транспортировки и хранения более 6 часов кровь должна храниться при температуре 2 - 8 °C.
- Нельзя замораживать и размораживать образец крови.
- Для получения наилучших результатов образец крови должен быть свежим, т. е. между его взятием и анализом должно пройти не более шести часов.
- Образцы крови можно использовать в течение 24 часов после взятия, если они хранятся в холодильнике при температуре 2 - 8 °C.
- Перед началом анализа пробирку с образцом крови следует аккуратно перевернуть от 8 - 10 раз.
- Использование тромбов запрещено.

11 Процедура анализа

ПРИМЕЧАНИЕ *Обратное пипетирование крайне важно для обеспечения точности, особенно при дозировании проб очень малого объёма. Рекомендуется использовать калиброванную электронную пипетку, запрограммированную на работу в режиме обратного пипетирования.*
Если электронная пипетка недоступна, выполните обратное пипетирование вручную следующим образом:
- *нажмите на кнопку управления до второго упора. Дайте кнопке управления полностью подняться, при этом избыток пробы втягивается в кончик.*
- *нажмите на кнопку управления до первого упора, чтобы выдавить точный объём крови, оставив избыток пробы в кончике.*
- *Выбросьте кончик пипетки с избытком пробы крови в контейнер для медицинских отходов.*

Обратите внимание, что обратное пипетирование нельзя использовать для растворов антител и буферных растворов.

11.1 Окрашивание
Переверните пробирку с ЭДТА и образцом крови примерно 8 - 10 раз.
Возьмите пипеткой 20 мкл образца цельной крови с ЭДТА со дна пробирки, не оставляя наконечником пипетки потеков крови на стенке пробирки. Отбросьте наконечник пипетки.
Добавьте 10 мкл раствора антитела CD4 mAb PE непосредственно в образец крови и аккуратноперемешайте, избегая образования потеков крови на стенке пробирки. Отбросьте наконечник пипетки.
Добавьте 10 мкл раствора антитела CD45 mAb PE-Cy5 непосредственно в образец крови и аккуратно перемешайте, избегая образования потеков крови на стенке пробирки. Отбросьте наконечник пипетки.
Инкубируйте смесь в течение 15 минут при температуре от 15 - 30 °C в темноте.
Добавьте 400 мкл буфера 1 и встряхните образец или аккуратно перемешайте вихревыми движениями. Отбросьте наконечник пипетки.
Добавьте 400 мкл буфера 2 и встряхните образец или аккуратно перемешайте вихревыми движениями. Отбросьте наконечник пипетки.
Непосредственно перед анализом с помощью цитометра CyFlow™ Counter образец следует встряхнуть или аккуратно перемешать вихревыми движениями.

ПРИМЕЧАНИЕ *После добавления буфера 1 образцы можно хранить не более 2 часов при температуре 2 - 8 °C в темном месте. Не используйте образцы, хранившиеся более длительное время или неправильно приготовленные.*
После добавления буфера 2 образцы должны быть проанализированы в течение 10 минут.

11.2 Анализ образца
Пожалуйста, обратитесь к IFU CyFlow™ Counter о том, как работать с прибором Перед анализом образцов. Процедуры запуска и внутренняя процедура контроля качества должны быть успешно завершено перед анализом образца.
- Выберите и загрузите конфигурацию для измерения CD4% в строке меню CyFlow™ Counter.
- Вставьте пробирку с приготовленным образцом крови в соответствующий держатель.
- Начните анализ.
- После анализа образца прибор прекращает работу и очищается автоматически.
- Извлеките и утилизируйте образец крови в соответствии с местными правилами по биологической безопасности при работе в лабораториях.

Не проводите повторный анализ с использованием той же самой пробирки с образцом. Если нужно выполнить дублирующее или повторное измерение, вторую пробирку для анализа подготовьте согласно п 11.1.

11.3 Сбор и анализ данных
Анализ данных с помощью цитометра CyFlow™ Counter возможен только при успешном выполнении процедуры внутреннего контроля качества прибора.
Информация, необходимая для запуска и технического обслуживания прибора, содержится в ИЭ цитометра CyFlow™ Counter (CY-S-3022IFUEN).
На экране появится результат подсчета CD4 т-клеток, процент CD4 и общее количество лимфоцитов.

Рисунок 1: Пример хорошего отделения кластеров гранулоцитов и других положительно окрашенных CD45-лейкоцитов от шумовых сигналов.

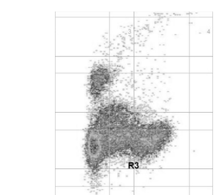

Рисунок 2: Пример плохого разделения мусора и положительных окрашенных лейкоцитов CD45. Этот образец не анализируемых.

Результаты будут неверными, если позиционирование ворот положительных окрашенных клеток CD45 (R3) выполняется точно.

12 Расчет результатов анализа
Расчет концентрации клеток выполняется в рамках анализа данных программным обеспечением, что дает результат определения показателя CD4% и количества клеток CD4+ на 1 мкл образца крови, а также количества лимфоцитов на 1 мкл образца крови после каждого анализа.
Для получения дополнительной информации см. ИЭ цитометра CyFlow™ Counter (CY-S-3022IFUEN).

13 Интерпретация результатов
Вирус иммунодефицита человека (ВИЧ) является одной из основных причин истощения клеток CD4, которая ослабляет иммунную систему. Результаты должны интерпретироваться врачами в сочетании с местными рекомендациями по лечению ВИЧ.

14 Контрольная процедура
Следует проводить надлежащий контроль качества согласно местным и национальным нормативным требованиям.
Существуют различные типы стабилизированного контроля крови образцы доступны на розничном рынке. Не все подходят для каждого потока цитометре. Вы можете узнать, какой контроль крови вы используете на вашем Sysmex представителя. Если критерии приемки не выполнены после измерения подходящей контрольной пробы крови, не приступать к анализу образцов пациента.

15 Рабочие характеристики
15.1 Специфичность
Антитело MEM-241 распознает CD4-антиген, трансмембранный гликопротеин массой 55 кДа, экспрессируемый подгруппой Т-лимфоцитов (Т-клетки «хелперы»), а также моноцитами, тканевыми макрофагами и гранулоцитами. Рабочее совещание по дифференцировочным молекулам клеток человека — HCDM (ранее VIII семинар по дифференцировочным антигенам лейкоцитов человека — HLDA), май 2006 г., Квебек, Канада; код WS M241
Антитело MEM-28 взаимодействует со всеми альтернативными формами человеческого антигена CD45 (общий лейкоцитарный антиген) — одноцепочечного трансмембранного белка типа I массой 180–220 кДа с высоким уровнем экспрессии на поверхности всех клеток гемопоэтического происхождения, за исключением эритроцитов и тромбоцитов. III семинар HLDA; код WS NL 833a.

15.2 Способность обнаружения
Выполнялась оценка для предела холостой пробы (LoB), предела обнаружения (LoD) и предела количественного определения (LoQ) согласно спецификациям, указанным в руководстве CLSI EP17-A2.
LoB: ≤50% of LoD
LoD: ≤20 CD4 клеток/мкл
LoQ: ≤40 CD4 клеток/мкл

15.3 Воспроизводимость
Вариабельность в пределах одного и нескольких анализов:
Значения коэффициента вариации (CV) составляют менее 10 % для количества CD4-клеток, равного или превышающего 200 CD4-Т-клеток на 1 мкл.
Значения коэффициента вариации (CV) для значений CD4% ≥ 25% ниже 10%.
Значения коэффициента вариации (CV) составляют менее 15 % для абсолютного количества CD4-клеток ниже 200 CD4-Т-клеток на 1 мкл.
Значения стандартного отклонения (SD) ≤ 2,5% для значений CD4% ниже 25%.

15.4 Линейность
Диапазон измерений анализа и линейность анализа валидны в пределах следующего диапазона:
абсолютное количество CD4-клеток:40-2500 CD4-Т-клеток на 1 мкл
CD4%: 4 – 60%

15.5 Правильность
Образцы цельной крови с ЭДТА окрашивали с помощью набора CD4% easy count kit и анализировали на цитометре CyFlow™ Counter (CyView™ 2.11). Абсолютное количество и процентное содержание CD4-клеток сравнивали с результатами анализа на цитометре BD FACSCalibur с использованием реагентов BD Tritest CD3/CD4/CD45 и пробирок BD Trucount. Систематическое расхождение между методами составило
-22,56 % (абсолютное количество CD4-клеток) и +2,1 % (CD4%).

16 Ограничения

ВНИМАНИЕ ⚠ *Наборы Sysmex Partec CD4% easy count (05-8405) не были валидированы для использования у пациентов детского и подросткового возраста.*

ВНИМАНИЕ ⚠ *Измерения CD4 и CD4%, сделанные с использованием Sysmex партек испытаний, не должны использоваться наряду с методами обнаружения CD4 и CD4% от других производителей. Используйте тесты только с Sysmex Партек устройствами. Значения, полученные с помощью других инструментов или анализов, не взаимозаменяемы.*

Различные эндогенные и экзогенные вещества были проверены в соответствии с руководством CLSI EP07-A2 на возможность искажения результатов анализа образца крови. В ≥ 18 % случаев гемолиза (вызванного применением протокола, рекомендованного в CLSI EP07-A2, приложение G, G1: метод осмотического шока) наблюдалось искажение результатов. По этой причине не следует использовать гемолизированные образцы. Для определения CD4 и CD4 в целом образцы крови с CyFlow™ Counter анализы, ни один другой антикоагулянт, чем ЭДТА должна быть использована. При наличии дополнительных вопросов о характеристиках продукта обратитесь к местному представителю компании Sysmex.

17 Литература

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18 Контактная информация производителя

Производитель
Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz, Германия
www.sysmex-partec.com
Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com

19 Номер редакции и дата выпуска ИЭ

Редакция: ред-07_10-09-2020_CN 1563
Выпущено:: Sysmex Partec GmbH

Паспорт безопасности данного продукта доступен на веб-сайте www.sysmex-partec.com/services.

1.4 CD4% easy count kit IFU (product code 05-8411)

IVD CE 0197

REF 05-8411

CD4% easy count kit

EN

Read and follow instructions carefully.
Note: Changes to previous version highlighted

1 Identification of the IVD reagent

Name	CD4% easy count kit	
Ref. No.	05-8411	
UDI-DI	04250878904849	
Content	Vial containing CD4 mAb PE	05-8411-01
	Vial containing CD45 mAb PE-Cy5	05-8411-02
	Bottle containing Buffer 1	05-8411-03
	Bottle containing Buffer 2	05-8411-04
	All components are ready to use.	

2 Specification

Specificity	Human CD4	Human CD45
Isotype	IgG1	IgG1
Clone	Mouse MEM-241	Mouse MEM-28
Content	100 tests	100 tests
Fluorochrome	PE	PE-Cy5
λ excitation (nm)	496 / 566	565
Emission maximum (nm)	576	666

3 Intended purpose

IVD For In Vitro Diagnostic Use.

The CD4% easy count kit is a four-component, quantitative IVD test for labelling of leukocytes and a subpopulation of lymphocytes in adult venous EDTA whole blood, which can then be enumerated with a suitable Sysmex Partec IVD flow cytometer after manual sample preparation. The CD4 T cell concentration and CD4% of lymphocytes in blood samples are useful to assess the immune and clinical status of patients. They are indicators for the initiation or follow-up of treatment for people living with HIV, in conjunction with other laboratory and clinical findings. The test is intended to be performed by trained healthcare professionals.

4 Principle of the procedure

An aliquot of an EDTA whole blood sample is mixed with two antibodies (CD4 and CD45), each conjugated to a different fluorochrome for labelling dedicated cell populations. After a fixed incubation time, the two buffer solutions are added, and the sample is ready for analysis e.g., on the CyFlow™ Counter flow cytometer. The light source excites the fluorescent dye linked with the stained cell and the emitted light is detected while a precise volume of blood sample is running through the instrument. The concentration of the dedicated cell populations is calculated by the integrated software.

For further information, please refer to the instructions for use (IFU) of the suitable Sysmex Partec IVD flow cytometer.

5 Storage and shelf life

5.1 Unopened product

Store the antibody and buffer reagents at 2 - 8°C in the dark. Do not freeze or expose the reagents to elevated temperatures and keep them away from direct sunlight. Under these conditions the reagent kit will be stable until the expiration date printed on the label. Do not use the reagents after the expiration date.

5.2 Product after first opening

Always close the bottle after use and use new pipette tips each time the reagent is sampled to avoid contamination. The shelf life after first opening is the same as the shelf life for unopened reagents if stored at stated storage conditions and used according to the instructions above.

6 Components

CD4 mAb PE and CD45 mAb PE-Cy5 are murine monoclonal antibodies supplied in PBS buffer with 0.2% BSA and 0.09% sodium azide. Buffer 1 and Buffer 2 are PBS-based solutions containing 0.09% sodium azide.

7 Evidence of deterioration

The antibody and buffer solutions are clear liquids. Do not use the reagents after appearance of any kind of turbidity or contamination.

For questions regarding the performance or quality of the product, please contact your local Sysmex representative.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user is located.

8 Precautions and warnings

Reagents contain 0.09% sodium azide as a preservative. The low concentration of sodium azide does not require hazard labelling, but the normal safety precautions for the handling of chemicals must be observed. Refer to the Safety Data Sheet (SDS) for a complete list of warnings and precautions. Safety Data Sheet for this product is available at http://www.sysmex-partec.com/services, or at https://us.sysmex-flowcytometry.com/.

9 Additional required equipment

Instrument:	Suitable Sysmex Partec IVD flow cytometer, i.e., CyFlow™ Counter (Ref. No. CY-S-3022 or CY-S-3023)
Laboratory equipment:	Venous blood collection system with EDTA (K2 or K3) as anticoagulant
	Calibrated pipettes with disposable pipette tips for 10, 20 and 100-1000 µL
	Sample tube(s) compatible with the flow cytometer
	Adequate personal protective equipment
	Stopwatch

Other materials may be required. Refer to the appropriate flow cytometer IFU for more information.

10 Disposal

Dispose of product after the expiration date in accordance with local regulations.

11 Primary sample collection, handling and storage

WARNING *All biological specimens and materials are considered biohazards and should be handled as if capable of transmitting infection. Appropriate safety precautions and handling procedures must be applied in accordance with applicable laws and regulations.*

- The blood sample must be a venous, whole-blood sample from a human adult, collected with a blood collection system which contains EDTA (K2 or K3) as anticoagulant.
- The blood sample must be transported in a container, protected from light and not exposed to elevated temperatures (e.g. a polystyrene box with cooling material).
- For best conditions, use fresh blood samples.
- Blood samples can be used up to 24 hours after drawing if they are stored at 2 - 8°C. Blood samples that have been transported and stored at up to 30°C must be analysed within 6 hours after drawing.
- Do not freeze and thaw the blood sample.
- Before use, gently invert the sample tubes containing the blood 8 to 10 times.
- Do not use clotted blood samples.

12 Examination procedure

NOTE *Reverse pipetting is critical to accuracy, especially when dispensing viscous and very small sample volumes. For whole blood pipetting, we recommend using a calibrated electronic pipette which is preprogrammed to operate in the reverse pipetting mode. If an electronic pipette is not available, follow these instructions for manual reverse pipetting:*

- *Depress the operating button to the second stop and place it in the homogenised specimen. Let the operating button move up completely. Excess sample is drawn up into the tip.*
- *Depress the operating button to the first stop to expel a precise volume of blood, leaving excess sample in the tip.*
- *Discard pipette tip with excess blood sample in the tip into medical waste container.*

Do not use reverse pipetting for antibody or buffer solutions.

12.1 Quality control procedure

The CD4% easy count kit can be used without a biological control material. For information on the quality control procedure for the Sysmex Partec IVD flow cytometer, please refer to the corresponding instructions for use.

12.2 Staining

1. Invert the blood sample in the EDTA blood collection tube gently 8 to 10 times.
2. Pipette 20 µL EDTA whole blood sample to the bottom of an unused sample tube. Avoid leaving a trail of blood from the pipette tip on the inner tube wall. Discard the pipette tip.
3. Add 10 µL CD4 mAb PE directly into the blood sample and mix it gently. Avoid leaving a trail of blood from the pipette tip on the inner tube wall. Discard the pipette tip.
4. Add 10 µL CD45 mAb PE-Cy5 directly into the blood sample and mix it gently. Avoid leaving a trail of blood from the pipette tip on the inner tube wall. Discard the pipette tip.
5. Incubate the mixture for a minimum of 15 minutes and a maximum of 30 minutes at 15 - 30°C in the dark.
6. Add 400 µL Buffer 1 and vortex briefly or tap tube gently to mix the sample. Discard the pipette tip.
7. Add 400 µL Buffer 2 and vortex briefly or mix the sample by pipetting up and down. Discard the pipette tip.
8. Proceed with sample analysis immediately.

NOTE *After addition of Buffer 1, samples can be stored for up to 2 hours at 2 - 8°C in the dark.*

After addition of Buffer 2, samples must be analysed within 10 minutes. Briefly vortex or mix the sample again by pipetting up and down immediately before analysis.

Do not use samples that have exceeded maximum storage time.

12.3 Sample analysis

Please refer to the IFU of the Sysmex Partec IVD flow cytometer for how to operate the instrument. The start-up procedures and the internal quality control procedure must be successfully completed before sample analysis. The following steps describe the sample analysis on a suitable CyFlow™ Counter.

1. Choose and load the configuration for CD4% measurement from the menu bar of the CyFlow™ Counter.
2. Insert the sample tube with the prepared blood sample into the sample port.
3. Start the measurement.
   After the sample run, the instrument stops and cleans automatically.
4. Remove the sample tube from the sample port and dispose of tube and remaining blood sample in accordance with local laboratory biohazard safety procedures.

Do not start a second measurement with the leftovers in the sample tube. If a duplicate or repeat measurement is required, use a new sample tube, and prepare a fresh sample.

12.4 Data acquisition and analysis

The following steps describe the data acquisition and analysis on a suitable CyFlow™ Counter.

Data analysis with the CyFlow™ Counter should only be performed after the internal quality control of the instrument was successful.

Please refer to the IFU of the CyFlow™ Counter for start-up procedure and maintenance of the device, if necessary.

The CD4 T cell counting result, CD4 percentage and lymphocyte concentration will be displayed on the screen. For results to be reliable there must be good separation of cell clusters as shown in Figure 1. If the clusters of stained cells towards the debris signals in the CD45 – SSC dot plot are not clearly separable (see Figure 2), or the clusters of CD4 positive cells, CD4 negative cells and monocytes in the CD4 – SSC dot plot are not clearly separable, the sample preparation and measurement must be repeated.

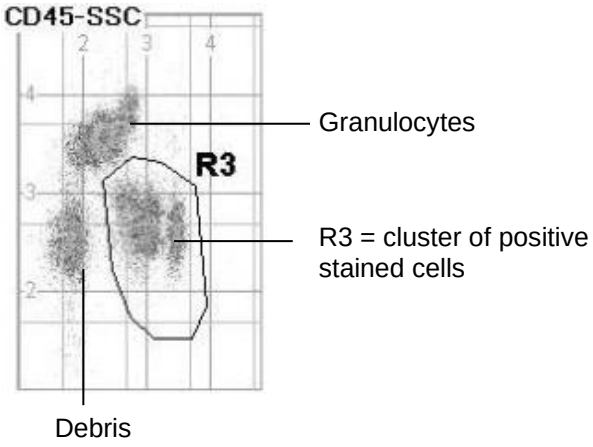

Figure 1: Example of well separated clusters of granulocytes, other CD45 positive stained leucocytes and debris signals.

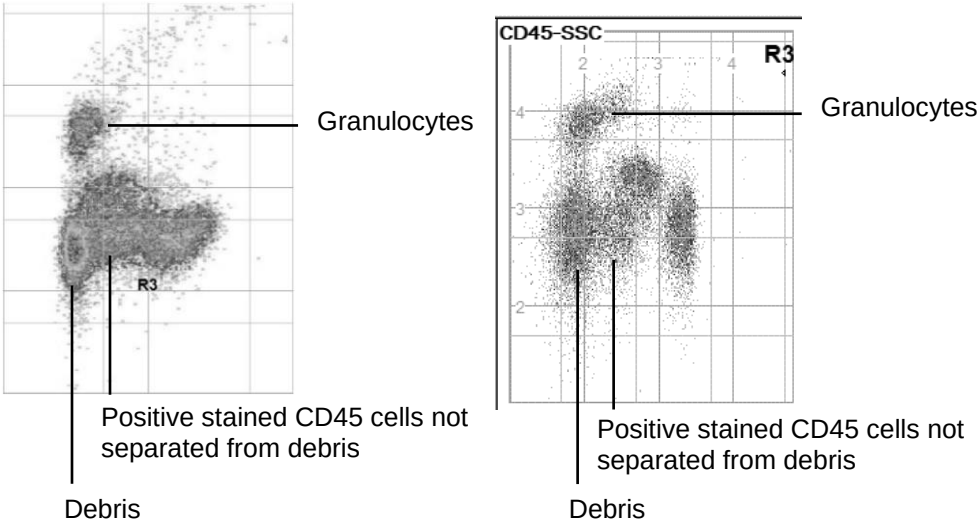

Figure 2: Examples of poor separation between debris and CD45 positive stained leucocytes. These samples are not analysable.

Results will be incorrect if gate positioning of CD45 positive stained cells (R3) is not done precisely.

13 Calculation of examination results

Calculation of the cell concentration is part of the software-based data analysis, which provides the result in CD4% values, and CD4 T cells per µL blood sample after each measurement. For further information, please refer to the IFU of the Sysmex Partec IVD flow cytometer, such as the CyFlow™ Counter.

14 Interpretation of results

Human Immunodeficiency Virus (HIV) is one of the main reasons for CD4 T cell depletion, which debilitates the immune system. The CD4 T cell concentration and CD4% of lymphocytes in blood samples are useful to assess the immune and clinical status of patients. They are indicators for the initiation or follow-up of treatment for people living with HIV, in conjunction with other laboratory and clinical findings. Results should be interpreted by physicians, in conjunction with locally applicable HIV treatment guidelines.

15 Analytical performance characteristics

15.1 Analytical sensitivity

Please refer to Limit of detection in section 15.4.

15.2 Analytical specificity

The clone MEM-241 recognises CD4 antigen, a 55kDa transmembrane glycoprotein expressed on a subset of T lymphocytes ("helper" T cells) and on monocytes, tissue macrophages and granulocytes. HCDM (former HLDA VIII) Meeting, May 2006, Québec, Canada; WS Code M241[1]

The clone MEM-28 reacts with all alternative forms of human CD45 antigen (Leukocyte Common Antigen), a 180-220kDa single chain type I transmembrane protein expressed at high level on all cells of hematopoietic origin, except erythrocytes and platelets. HLDA III; WS Code NL 833a[2]

15.3 Accuracy

Trueness/Bias

EDTA whole blood specimens were stained with CD4% easy count kit and analysed on the CyFlow™ Counter (CyView™ 2.11) at three test sites (n = 1021 total). CD4 absolute and CD4% values were compared with results from a BD FACSCalibur™ using BD Tritest™ or Multitest reagents and BD Trucount™ tubes and showed a mean bias of -18.71% (CD4 absolute, limits of agreement: -52.95%; +15.52%) and +0.82% (CD4%, limits of agreement: -29.46%; +31.10%) between both methods.

Precision

Tab. 1: Evaluation of repeatability for the CD4 and CD4% values of the CD4% easy count kit using low and normal blood controls.

Sample	N	CD4 value – Repeatability (SD)	CD4 value – Repeatability (%CV in %)	CD4% value – Repeatability (SD% in %)	CD4% value – Repeatability (%CV in %)
Low Blood Control (< 200 CD4 T cells/µL)	405	6.90	5.60	0.58	5.90
Normal Blood Control (≥ 200 CD4 T cells/µL)	404	44.30	4.80	0.79	1.80

SD = Standard Deviation %CV = Coefficient of Variation as a percentage

Tab. 2: Evaluation of reproducibility for the CD4 and CD4% values of the CD4% easy count kit using low and normal blood controls.

Sample	N	CD4 value – Reproducibility (SD)	CD4 value – Reproducibility (%CV in %)	CD4% value – Reproducibility (SD% in %)	CD4% value – Reproducibility (%CV in %)
Low Blood Control (< 25% CD4%)	225	8.30	6.70	0.98	11.13
Normal Blood Control (≥ 25% CD4%)	225	65.80	7.00	2.07	5.00

SD = Standard Deviation %CV = Coefficient of Variation as a percentage

15.4 Detection capabilities

The evaluation for Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) was carried out according to the specifications in the guideline CLSI EP17-A2.

LoB: 1 CD4 T cells/µL

LoD: 3 CD4 T cells/µL

LoQ: 20 CD4 T cells/µL

15.5 Measuring range/Linearity

For CD4 by using the polynomial method according to CLSI EP06-A, the method has been demonstrated to be linear from 40 to 2500 CD4 T cells/µL within 10% of the 1st order fit at > 200 CD4 T cells/µL and within ± 20 cells/µL at ≤ 200 CD4 T cells/µL in this interval.
For CD4% by using the polynomial method according to CLSI EP06-A, the method has been demonstrated to be linear for CD4% values from 4 to 60% within 10% of the 1st order fit at > 200 CD4 T cells/µL and within ± 20 cells/µL at ≤ 200 CD4 T cells/µL in this interval.

15.6 Assay cut-offs

The cut-offs are defined by the World Health Organization (WHO) and were initially specified as 200 cells/µL, later as 350 cells/µL and 500 cells/µL.[3][4][5] For managing Advanced HIV Disease (AHD) a cut-off of 200 cells/µL was set.[6] The clinical parameters were determined using the 3 cut-offs.

16 Clinical performance characteristics

16.1 Diagnostic sensitivity & specificity, positive & negative predictive value, likelihood ratio

Tab. 3: Evaluation of diagnostic parameters of blood specimens from HIV positive patients and other patients undergoing routine CD4 T+ cell enumeration from three sites for the CD4 values of the CD4% easy count kit with three different cut-offs, reference method: BD FACSCalibur™, n = 1021.

Cut-off [cells/ µL]	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	PLR (95% CI)	NLR (95% CI)
200	98.2	93.4	74.7	99.6	14.96	0.02
	(94.9 -99.4)	(91.6-94.9)	(68.5-79.9)	(98.9-99.9)	(11.61-19.29)	(0.01-0.06)
350	98.6	83.1	76.5	99.1	5.82	0.02
	(96.8-99.4)	(80.0-85.7)	(72.5-80.1)	(97.9-99.6)	(4.91-6.90)	(0.01-0.04)
500	99.7	69.5	81.2	99.4	3.27	0.01
	(98.8-99.9)	(65.1-73.7)	(78.2-83.9)	(97.7-99.8)	(2.84-3.77)	(0.01-0.02)

CI = Confidence Interval PPV = Positive Predictive Value NPV = Negative Predictive Value
PLR = Positive Likelihood Ratio NLR = Negative Likelihood Ratio

Tab. 4: Evaluation of diagnostic parameters of blood specimens from HIV positive patients and other patients undergoing routine CD4 T+ cell enumeration from three sites for the CD4% values of the CD4% easy count kit with one cut-off, reference method: BD FACSCalibur™, n = 1021.

Cut-off [%]	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	PLR (95% CI)	NLR (95% CI)
25	96.2	96.3	97.2	95.0	26.17	0.04
	(94.4-97.5)	(94.1-97.7)	(95.6-98.3)	(92.6-96.7)	(16.17-42.34)	(0.03-0.06)

CI = Confidence Interval PPV = Positive Predictive Value NPV = Negative Predictive Value
PLR = Positive Likelihood Ratio NLR = Negative Likelihood Ratio

16.2 Expected values in normal and affected populations

Expected CD4 counts in healthy individuals with no HIV, in HIV positive patients under treatment or with low viral load: > 500 CD4 T cells/µL.[5] Cut-off values for HIV positive patients < 500 / 350 / 200 CD4 T cells/µL (see section 15.6).

17 Limitations

Patient measurements of CD4 and CD4% made using Sysmex Partec assays should not be used alongside measurements obtained from other manufacturer's methods of determining CD4 and CD4%. Use the assay only with a suitable Sysmex Partec IVD flow cytometer. Values obtained using other equipment or assays are not interchangeable.

Different endogenous and exogenous substances were tested according to the CLSI EP07-A2 guideline for possible interfering effects which could have an influence on the blood sample analysis. In summary, ≥ 18% haemolysis (induced by using the protocol recommended in the CLSI EP07-A2, Appendix G, G1 - Osmotic Shock Procedure) was observed to show an interfering effect. For that reason, haemolysed samples should be rejected. Excessive levels of white blood cells lead to increased CD4 concentrations.

The presence of antibodies in a patient's specimen (e.g., human anti-animal antibodies, rheumatoid factors, or therapeutic antibodies) can interfere with the principle of the examination procedure. If antibodies directly interfere with CD4 mAb PE and/or CD45 mAb PE-Cy5, this could result in spuriously low CD4 concentrations.[7][8]

The presence of endogenous proteins in a patient's specimen (e.g., factors of the complement system or enzymes) can interfere with the principle of the examination procedure. If endogenous proteins directly interfere with CD4 mAb PE and/or CD45 mAb PE-Cy5, this could result in spuriously low CD4 concentrations.[9]

Chronic smoking may lead to an increase in the number of leukocytes, which might lead to an over-estimation of cell counts.[10][11]

Accurate and reproducible results will be obtained if the procedures used are in accordance with the IFU and compatible with good laboratory practices. This includes the avoidance of contaminations from various sources such as sample collection and preparation material.

18 Literature

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19 Summary of safety and performance

The summary of safety and performance will be supplied in the Eudamed database.

20 Manufacturer

Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz
Germany

Tel +49 3581 8746 0
Fax +49 3581 8746 70
E-mail: info@sysmex-partec.com
www.sysmex-partec.com

21 Symbols

REF	Reference number		Manufacturer	LOT	Batch code
	Keep away from sunlight		Temperature limit	IVD	In vitro diagnostic medical device
	Use-by date		Consult instructions for use	CE	CE mark
Σ	Contains sufficient for <n> tests	CONTENT	Content of kit	UDI	Unique device identifier

22 Date of issue or revision

Rev.: 001
Rev. date: 13-03-2024
Doc. No.: 05-8411 IFU GB EN

CN 2148

1.5 Hypochlorite Solution IFU

1 Identification of the IVD reagent

Name	Hypochlorite Solution
Ref. No.	04-4019
UDI-DI	04250878904818
Content	250 mL, ready to use

2 Intended purpose

IVD For In Vitro Diagnostic Use.

Hypochlorite Solution is intended to clean the sample pathway by reducing possibly remaining blood and/or protein residuals from prior sample measurements in Sysmex Partec clinical flow cytometers. Hypochlorite Solution is ready to use and will be fed to the instrument via sample port manually or via automated loading system. Hypochlorite solution is an accessory solution and does not provide any diagnostic information.

Handling with Hypochlorite Solution is restricted to lab technicians and trained FCM operators.

3 Principle of the procedure

Hypochlorite solution, as an accessory solution for flow cytometry, is used to reduce possible residual proteins in the sample pathway of Sysmex Partec clinical flow cytometers after measurement.

For further information refer to the Instructions for Use of the flow cytometer.

4 Storage and shelf life

4.1 Unopened product

Store Hypochlorite Solution at 18-30 °C, do not freeze or expose to elevated temperatures. Under these storage conditions the reagent will be stable until the expiration date printed on its label. Do not use the reagent beyond its shelf life.

4.2 Product after first opening

The shelf life after first opening is the same as the shelf life for unopened reagent if stored at stated storage conditions and used according to the Instructions for Use (IFU).

5 Components

Hypochlorite Solution is an aqueous solution containing < 1.00 wt% of sodium hypochlorite (CAS no. 7681-52-9).

6 Evidence of deterioration

Hypochlorite Solution is a clear liquid. Do not use Hypochlorite Solution after appearance of any kind of turbidity or contamination.

For questions regarding to the performance or quality of the product received, please contact your local Sysmex representative.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the component authority of the Member State in which the user is located.

7 Precautions and warnings

Appropriate safety precautions and handling procedures must be applied in accordance with applicable laws and regulations.

Refer to the Safety Data Sheet (SDS) for a complete list of warnings and precautions.

7.1 Warning symbols

GHS05
Corrosive

7.2 Signal word

DANGER

7.3 Hazards

H314	Causes severe skin burns and eye damage.
H412	Harmful to aquatic life with long lasting effects.

7.4 Precautions

P260	Do not breathe mist/vapours/spray.
P264	Wash thoroughly after handling.
P280	Wear protective gloves/eye protection.
P303+P361+P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER/doctor.
P501	Dispose of contents/container to a facility in accordance with local and national regulations.

8 Additional required equipment

Instrument:	Sysmex Partec clinical flow cytometer, such as the CyFlow™ Counter (Ref. No. CY-S-3023)
	For further information, please refer to the instructions for use (IFU) of the flow cytometer.
Laboratory equipment:	A calibrated pipette and pipette tips
	Sample tube(s) compliant to the flow cytometer
	Personal protective equipment

9 Disposal

Disposal procedure should meet requirements of applicable local regulations.

10 Manufacturer

Sysmex Partec GmbH
Arndtstraße 11 a-b
02826 Görlitz
Germany

Phone +49 3581 8746 0
Fax +49 3581 8746 70
info@sysmex-partec.com
www.sysmex-partec.com

11 Symbols

REF	Reference number		Manufacturer	LOT	Batch code
	Temperature limit	IVD	In vitro diagnostic medical device		Use-by date
	Consult instructions for use	CE	CE-mark	UDI	Unique device identifier

12 Date of issue or revision

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