Mpox disease Emergency Use Listing Procedure (EUL) for IVDs
Product: Monkeypox Virus Lyo-PCR Kit
EUL Number: MPXV-13393-139-00
Outcome: Accepted.

The EUL process is intended to expedite the availability of in vitro diagnostics needed in public health emergency situations and to assist interested UN procurement agencies and Member States in determining the acceptability of using specific products in the context of a Public Health Emergency of International Concern (PHEIC), based on an essential set of available quality, safety, and performance data. The EUL procedure includes the following:

- Quality Management Systems Review and Plan for Post-Market Surveillance: a desktop review of the manufacturer's Quality Management System documentation and specific manufacturing documents.
- Product Dossier Review: assessment of the documentary evidence of safety and performance. This evaluation of limited scope is to verify critical analytical and performance characteristics.

The Monkeypox Virus Lyo-PCR Kit, with product codes W-ZD-0580-02-48A and W-ZD-0580-02-96A, Rest-of-World regulatory version manufactured by Shanghai ZJ Bio-Tech Co., Ltd, located at 1st Floor, Gate B, Building #20 & 1st Floor, Gate A, Building #21, 528 Ruiqing Road, Zhangjiang High-Tech Industrial East District, Shanghai 201203, People's Republic of China, was listed as eligible for WHO procurement on 17 September 2025.

Intended use:

According to the claim of intended use from Shanghai ZJ Bio-Tech Co., Ltd., *"The Monkeypox Virus Lyo-PCR Kit is a real-time PCR test intended for the qualitative detection of DNA from monkeypox virus (Clade I/II) in human lesion swab specimens (i.e., swabs of human pustular or vesicular rash) from individuals suspected of monkeypox virus infection by their healthcare providers.*
The results are used to aid in the diagnosis of MPXV (Clade I/II) infection. The monkeypox virus DNA is generally detectable in human lesion swab specimens (i.e., swabs of human pustular or vesicular rash) during the acute phase of infection. Positive results are indicative of the presence of monkeypox virus DNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results obtained with this device do not preclude monkeypox virus infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history and epidemiological information.

The kit is intended only for the detection of the monkeypox virus and is not applicable for detecting other orthopoxviruses. It is designed to target the monkeypox virus genes F3L and

B7R. The kit does not distinguish between Clade I and Clade II. The Monkeypox Virus Lyo-PCR Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of PCR and in vitro diagnostic procedures. Testing is limited to laboratories that comply with local regulatory requirements for MPXV."

Validated specimen type:
Human skin lesion swabs collected using standard nylon-flocked swab and

immediately into a sterile transport tube containing Viral Transport Medium (Shenzhen Zijian Biotechnology Co., LTD) or Noble Biosciences Clinical Virus Transport Medium with NS-1 Swab Applicator (cat.#UTNS-1C, standard nylon-flocked swab with a breakpoint), or in Universal Transport Medium (RPMI 1640 Medium (GIBCO (Life Technologies Corporation), cat.#11875119.

Test kit contents:

ref	*Type of reagent*	*Quantity sufficient for 48 Rxns*	*Quantity sufficient for 96 Rxns*
1	MPXV PCR Reaction Tube	48 tubes	96 tubes
2	MPXV Positive Control	3 bags, 1 vial dry powder/bag	6 bags, 1 vial dry powder/bag
3	MPXV Negative Control	3 tubes, 400µL/tube	6 tubes, 400µL/tube
4	Molecular Grade Water	3 tubes, 1,000µL/tube	6 tubes, 1,000µL/tube

Items required but not provided:

DNA Extraction Options

Extraction method 1
Materials required in the following: -Roche MagNA Pure 96 Instrument (Roche, catalog # 06541089001) -MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche, catalog #06543588001) -MagNA Pure 96 System Fluid (Roche, catalog #06430112001) MagNA Pure 96 External Lysis Buffer (Roche, catalog #06374913001)
Extraction method 2
Materials required in the following: -Liferiver AutoEX36 E Extraction Kit (SHANGHAI ZJ BIO-TECH CO., LTD, catalog #ME-0042) -Automated Nucleic Acid Extraction Instrument (EX3600) (Shanghai ZJ Bio-Tech Co., Ltd.)
Extraction method 3
Materials required in the following: -Viral DNA/RNA Isolation Kit (Centrifuge Column) (SHANGHAI ZJ BIO-TECH CO., LTD, catalog #ME-0078) -Ethanol (96–100%) -Heating block for lysis of samples at 56°C -Microcentrifuge (with rotor for 1.5ml and 2ml tubes as well as speed of 6,000 x g (8,000 rpm) and 20,000 x g (14,000 rpm)) or equivalent

Other equipment and consumables required but not provided:

- Vortexmixer (Qinlinbeier, catalog # VORTEX-5) or equivalent
- Palm centrifuge (Qinlinbeier, catalog # LX-200) or equivalent
- Micropipettes (10µL/100µL/1,000µL)
- Racks for 1.5mL microcentrifuge tubes
- Real-time PCR instrument: Applied Biosystems 7500 (Software Version 2.3) or Life9600 Real-Time PCR System (Software Version LifeQP 1.2.1)
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach) 75% ethanol
- DNAzap (Ambion, cat. #AM9890) or equivalent
- Disposable powder-free gloves and surgical gowns
- Aerosol barrier pipette tips (10µL/100µL/1,000µL)
- 1.5mL microcentrifuge tubes (Axygen, catalog #MCT-150-C) or equivalent
- Standard nylon-flocked swab and
- VTM: Disposable virus sampling tube (containing virus storage solution) (Shenzhen Zijian Biotechnology Co., LTD); or
- UTM: RPMI 1640 Medium (GIBCO (Life Technologies Corporation), cat.#11875119); or VTM: Noble Biosciences Clinical Virus Transport Medium with NS-1 Swab Applicator (cat.#UTNS-1C, standard nylon-flocked swab with a breakpoint)

Storage:
The test kit must be stored at 2-30 °C in a dry place and kept away from light.

Shelf-life upon manufacture:
The shelf-life is currently assigned 18 months dating.

Warnings/limitations:

Please refer to the Instructions for Use attached to this public report.

Product dossier assessment

Shanghai ZJ Bio-Tech Co., Ltd. submitted the product dossier for the Monkeypox Virus Lyo-PCR Kit in alignment with the Instructions and requirements for Emergency Use Listing (EUL) Submission: In vitro diagnostics detecting Monkeypox virus nucleic acid (PQDx_457). The WHO reviewed the information provided in the dossier.

The risk-benefit assessment conclusion was acceptable.

Quality Management Systems Review

To establish eligibility for WHO procurement, Shanghai ZJ Bio-Tech Co., Ltd. was asked to provide up-to-date information about the status of its quality management system.

Based on the WHO's review of the submitted quality management system documentation, Shanghai ZJ Bio-Tech Co., Ltd. provided sufficient information to fulfil the requirements described in the Instructions and requirements for EUL Submission: In vitro diagnostics detecting Monkeypox virus nucleic acid (PQDx_457).

The conclusion of the quality management system assessment was acceptable.

Plan for Post-Market Surveillance

Post-market surveillance, including monitoring all customer feedback, detecting and acting on adverse events, product problems, non-conforming goods and processes is a critical component of minimising the potential harm of an IVD listed for emergency use.

The following post-EUL activities are required to maintain the EUL status:

1. Notification to WHO of any planned changes to a prequalified product, in accordance with "*Reportable changes to WHO prequalified and emergency use listed in vitro diagnostics*"[1]; and
2. Post-market surveillance activities, in accordance with "*WHO guidance on post-market surveillance of in vitro diagnostics" (ISBN 978 92 4 150921 3)*[2].

Shanghai ZJ Bio-Tech Co., Ltd. is also required to submit an annual report summarising sales data and all complaints. Certain complaints and changes to the product must be notified immediately to WHO, as per the above-mentioned documents. The sales data will serve as denominator data to guide the frequency of re-inspection.

The manufacturer has committed to ensuring that post-emergency use listing safety, quality, and performance monitoring activities are in place, which are in accordance with WHO guidance on post-market surveillance of in vitro diagnostics.

Scope and duration of procurement eligibility

The Monkeypox Virus Lyo-PCR Kit, with product codes W-ZD-0580-02-48A and W-ZD-0580-02-96A, manufactured by Shanghai ZJ Bio-Tech Co., Ltd., is eligible for WHO procurement for 12 months from the day of listing. The assay detects nucleic acid of the monkeypox virus, including Clade I and Clade II. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO-prequalified. As part of the ongoing requirements for listing as eligible for WHO procurement, Shanghai ZJ Bio-Tech Co., Ltd. must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality, and performance requirements. Shanghai ZJ Bio-Tech Co., Ltd. is required to notify WHO of any complaints, including adverse events related to the use of the product, within 10 days.

[1] https://iris.who.int/handle/10665/381373

[2] https://iris.who.int/handle/10665/337551

WHO reserves the right to rescind eligibility for WHO procurement if additional information on the safety, quality, and performance during post-market surveillance activities and if new data becomes available to WHO that changes the risk-benefit balance.

Labelling

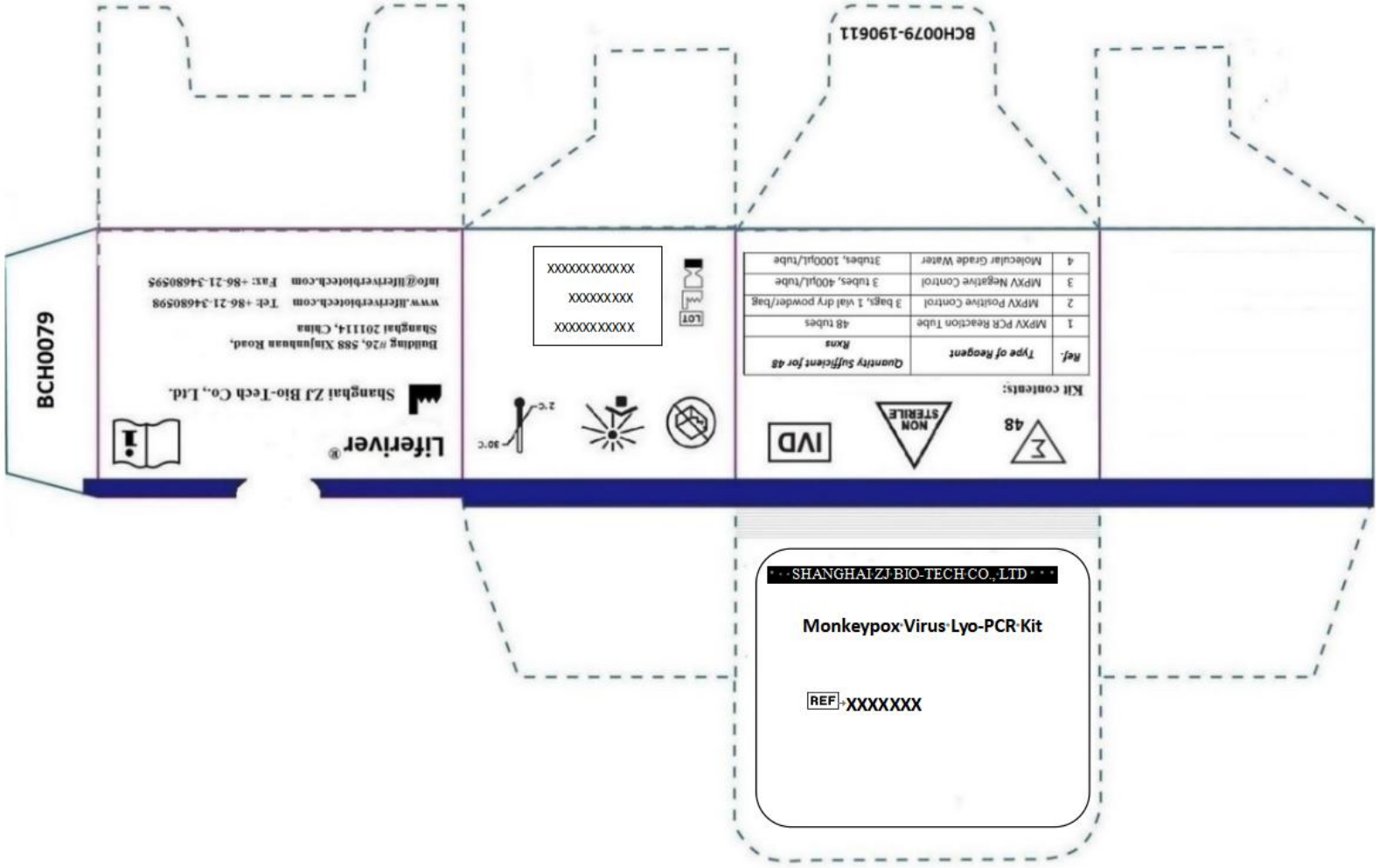
1. **Labels**
2. **Instructions for use**

1.0 Labels

Labels of Monkeypox Virus Lyo-PCR Kit

1. Labels of 48 Rxns

1.1 Labels for package



1.2 Labels for aluminum foil bags

MPXV PCR Reaction Tube
16 tubes per bag
Before opening the foil pouches: Store at 2-30°C in a dry place and keep away from light.
For dissolved dry powder reagents, Use immediately.

LOT XXXXXXXXXXX
XXXXXXXXXXXXX

×3

MPXV Positive Control
1 vial dry powder
Positive Control should be dissolved and mixed by adding 400µL Molecular Grade Water and vortexing before use.
Before opening the foil pouches: Store at 2-30°C in a dry place and keep away from light.
For dissolved dry powder reagents,Use immediately and discard any remaining liquid.

LOT XXXXXXXXXXX
XXXXXXXXXXXXX

×3

1.3 Labels for tubes

MPXV Positive Control
1 vial dry powder

LOT XXXXXXXXX
XXXXXXXXXX

×3

MPXV Negative Control
Vol：400µL
store at: 2-30°C

LOT XXXXXXXXXXX
XXXXXXXXXXXXX

×3

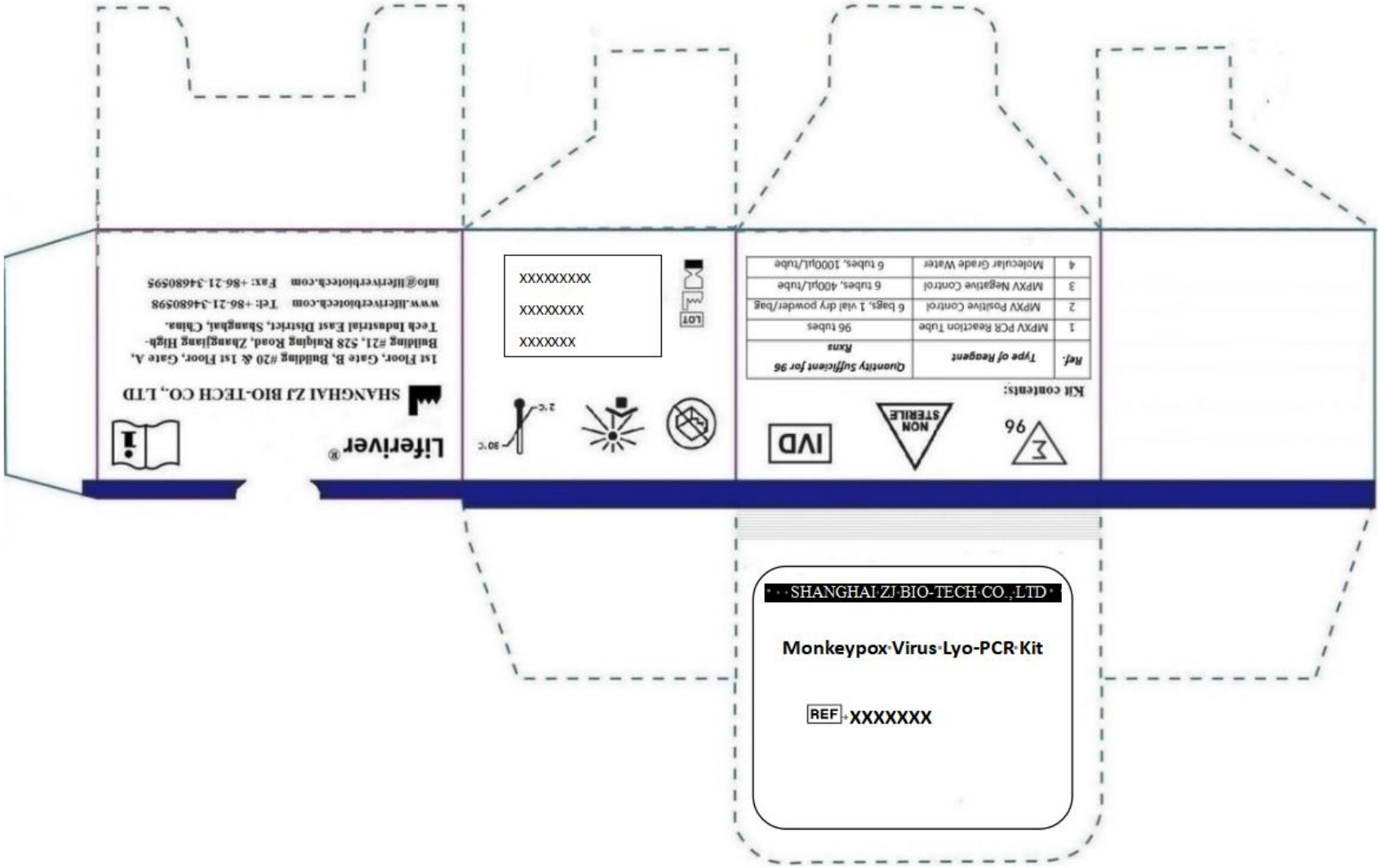
Molecular Grade Water
Vol: 1000µL
store at: 2-30°C

LOT XXXXXXXXXXX
XXXXXXXXXXXXX

×3

2. Labels of 96 Rxns

2.1 Labels for package



2.2 Labels for aluminum foil bags

MPXV PCR Reaction Tube
16 tubes per bag
Before opening the foil pouches: Store at 2-30°C in a dry place and keep away from light.
For dissolved dry powder reagents, Use immediately.

LOT XXXXXXXXXXX
XXXXXXXXXXXXX

×6

MPXV Positive Control
1 vial dry powder
Positive Control should be dissolved and mixed by adding 400µL Molecular Grade Water and vortexing before use.
Before opening the foil pouches: Store at 2-30°C in a dry place and keep away from light.
For dissolved dry powder reagents , Use immediately and discard any remaining liquid.

LOT XXXXXXXXXXX
XXXXXXXXXXXXX

×6

2.3 Labels for tubes

MPXV Positive Control
1 vial dry powder

LOT XXXXXXXXXXX
XXXXXXXXXXXXX

×6

MPXV Negative Control
Vol：400µL
store at: 2-30°C

LOT XXXXXXXXXXX
XXXXXXXXXXXXX

×6

Molecular Grade Water
Vol: 1000µL
store at: 2-30°C

LOT XXXXXXXXXXX
XXXXXXXXXXXXX

×6

2.0 Instructions for Use[3]

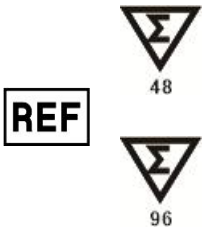
[3] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

Liferiver®

Monkeypox Virus Lyo-PCR Kit

Instructions For Use

For in vitro diagnostic use



W-ZD-0580-02-48A

W-ZD-0580-02-96A

For use with Applied Biosystems 7500 Real-Time PCR System or Life9600 Real-Time PCR System

SHANGHAI ZJ BIO-TECH CO., LTD
1st Floor, Gate B, Building #20 & 1st Floor, Gate A, Building #21, 528 Ruiqing Road, Zhangjiang High-Tech Industrial East District, Shanghai 201203, China.
www.liferiverbiotech.com Tel: +86-21-34680598
info@liferiverbiotech.com Fax: +86-21-34680595

Table of Contents

Intended Use

The Monkeypox Virus Lyo-PCR Kit is a real-time PCR test intended for the qualitative detection of DNA from monkeypox virus (Clade I/II) in human lesion swab specimens (i.e., swabs of human pustular or vesicular rash) from individuals suspected of monkeypox virus infection by their healthcare providers.

The results are used to aid in the diagnosis of MPXV (Clade I/II) infection. The monkeypox virus DNA is generally detectable in human lesion swab specimens (i.e., swabs of human pustular or vesicular rash) during the acute phase of infection[2]. Positive results are indicative of the presence of monkeypox virus DNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results obtained with this device do not preclude monkeypox virus infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history and epidemiological information [6].

The kit is intended only for the detection of the monkeypox virus and is not applicable for detecting other orthopoxviruses. It is designed to target the monkeypox virus genes F3L and B7R. The kit does not distinguish between Clade I and Clade II.

The Monkeypox Virus Lyo-PCR Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of PCR and in vitro diagnostic procedures. Testing is limited to laboratories that comply with local regulatory requirements for MPXV.

Summary and Explanation of the Test

Monkeypox is a rare disease caused by infection with the monkeypox virus (MPXV). Monkeypox virus is an enveloped double-stranded DNA virus with a rectangular shape that can grow in African green monkey kidney cells and cause cytopathic changes. Monkeypox virus is part of the same family of viruses as variola virus, the virus that causes smallpox[3].

The Monkeypox Virus Lyo-PCR Kit is a molecular in vitro diagnostic test that aids in the detection and diagnosis of monkeypox virus and is based on widely used real time PCR technology utilizing nucleic acid amplification technology. The product contains MPXV PCR Reaction Tube, control materials and Molecular Grade Water: The MPXV PCR Reaction Tube contains enzymes, primers and probes, buffer, and Mg2+ which are lyophilized and packaged in a tube; the control materials include positive control and negative control; the positive control is in lyophilized form and needs to be reconstituted with Molecular Grade Water before use.

Principles of the Procedure

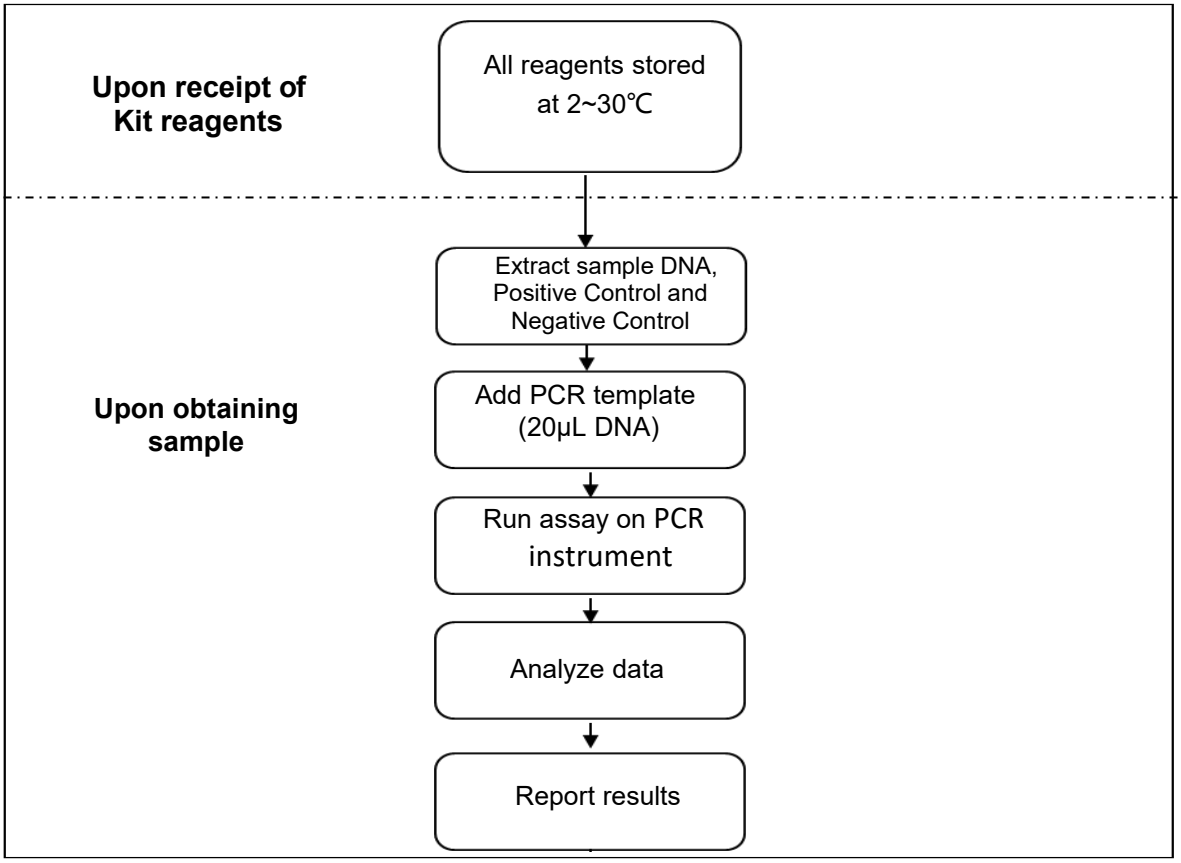
This kit employs a real-time quantitative PCR (qPCR) method based on TaqMan technology for the specific detection of Monkeypox virus (MPXV) DNA. The detection relies on multiple sets of primers and probes targeting the viral F3L/B7R genes and the human RNase P gene, with different fluorescent dyes used for

differentiation. The probe for MPXV detection is labeled with the FAM reporter group, while the probe for the internal control (human RNase P) is labeled with the Texas Red reporter group. During the PCR amplification process, Taq DNA polymerase hydrolyzes the probes bound to the template, causing the reporter group to separate from the quencher group and produce a fluorescent signal. The instrument collects the fluorescence intensity of each channel at the end of each cycle, thereby enabling real-time monitoring of the amplification process.

The interpretation of results depends on the analysis of amplification curves and Ct values (cycle threshold values). The Ct value is the number of cycles required for the fluorescence signal to reach a set threshold, which is inversely proportional to the initial template amount. If the Ct value in the MPXV detection channel (FAM) is below the preset cutoff value (Ct ≤ 42), the result is considered positive; if there is no amplification signal in the FAM channel and the Ct value in the internal standard channel is below the preset cutoff value (Ct ≤ 35), the result is negative. If there is no amplification signal in the FAM channel and the Ct value in the internal standard channel is above the preset cutoff value of 35 or there is no signal, it indicates that there is an issue with the sample or the reaction process, and the test result is invalid.

To ensure the accuracy of each test, controls must be set and results validated. The positive control contains known MPXV target and internal control sequences, and its positive amplification indicates that the reagents and instrument are functioning properly; the negative control should show no amplification in either the MPXV channel or the internal control channel, Any abnormal signal suggests possible contamination. Only when all control results meet expectations can the sample test results from that run be accepted.

Summary of Preparation and Testing Process



Reagents and Materials Provided

Ref.	*Type of Reagent*	*Quantity Sufficient for 48 Rxns*	*Quantity Sufficient for 96 Rxns*
1	MPXV PCR Reaction Tube	48 tubes	96 tubes
2	MPXV Positive Control	3 bags, 1 vial dry powder/bag	6 bags, 1 vial dry powder/bag
3	MPXV Negative Control	3 tubes, 400µL/tube	6 tubes, 400µL/tube
4	Molecular Grade Water	3 tubes, 1,000µL/tube	6 tubes, 1,000µL/tube

Control materials

- MPXV Positive Control is a mixture of plasmids containing partial monkeypox virus gene fragment and human RNase P gene which are designed to cover the target sequence and Internal Control sequence, The MPXV Positive Control can react with the reagent to indicate whether the reagent work normally.
- **MPXV Positive Control needs to be reconstituted with 400µL Molecular Grade Water before use.**
- MPXV Negative Control is 0.9%NaCl that serves as an external negative specimen during DNA extraction procedure.

Reagents and Materials Not Provided with the Test

DNA Extraction Options

The following extraction methods have been verified by experiments and are suitable for the Monkeypox Virus Lyo-PCR Kit:

Extraction method 1
Materials required in the following: ☑Roche MagNA Pure 96 Instrument (Roche, catalog # 06541089001) ☑MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche, catalog #06543588001) ☑MagNA Pure 96 System Fluid (Roche, catalog #06430112001) MagNA Pure 96 External Lysis Buffer (Roche, catalog #06374913001)
Extraction method 2
Materials required in the following: ☑Liferiver® AutoEX36 E Extraction Kit (SHANGHAI ZJ BIO-TECH CO., LTD, catalog #ME-0042) ☑Automated Nucleic Acid Extraction Instrument (EX3600) (Shanghai ZJ Bio-Tech Co., Ltd.)
Extraction method 3
Materials required in the following: ☑Viral DNA/RNA Isolation Kit (Centrifuge Column) (SHANGHAI ZJ BIO-TECH CO., LTD, catalog #ME-0078) ☑Ethanol (96–100%) ☑Heating block for lysis of samples at 56°C ☑Microcentrifuge (with rotor for 1.5ml and 2ml tubes as well as speed of 6,000 x g (8,000 rpm) and 20,000 x g (14,000 rpm)) or equivalent

Other Equipment and Consumables Required (Not Provided)

- Vortexmixer (Qinlinbeier, catalog # VORTEX-5) or equivalent
- Palm centrifuge (Qinlinbeier, catalog # LX-200) or equivalent
- Micropipettes (10µL/100µL/1,000µL)
- Racks for 1.5mL microcentrifuge tubes
- Real-time PCR instrument: Applied Biosystems 7500 (Software Version 2.3) or Life9600 Real-Time PCR System (Software Version LifeQP 1.2.1)
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- 75% ethanol
- DNAzap™ (Ambion, cat. #AM9890) or equivalent
- Disposable powder-free gloves and surgical gowns
- Aerosol barrier pipette tips (10µL/100µL/1,000µL)
- 1.5mL microcentrifuge tubes (Axygen, catalog #MCT-150-C) or equivalent
- Standard nylon-flocked swab and
- VTM: Disposable virus sampling tube (containing virus storage solution) (Shenzhen Zijian Biotechnology Co., LTD); or
- UTM: RPMI 1640 Medium (GIBCO (Life Technologies Corporation), cat.#11875119); or
- VTM: Noble Biosciences Clinical Virus Transport Medium with NS-1 Swab Applicator (cat.#UTNS-1C, standard nylon-flocked swab with a breakpoint)

Warnings and Precautions

- For in vitro diagnostic use.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, or apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious, following safe laboratory procedures.
- Specimen processing should be performed in accordance with national biological safety regulations[4].
- If infection with monkeypox virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions[5].
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC) in accordance with applicable WHO and national biosafety guidance[5].
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
    - Maintain separate areas for assay setup and handling of nucleic acids.

- Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different batches or from other manufacturers.
- Change aerosol barrier pipette tips between all manual liquid transfers.
- During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
- Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves between samples and whenever contamination is suspected.
- Keep reagent and reaction tubes capped or covered as much as possible.
- Do not open the reaction tubes/plates post amplification to avoid contamination with amplicons.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, DNA*Zap*™ to minimize risk of nucleic acid contamination. Residual bleach should be removed using 75% ethanol.

- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.
- Avoid the existence of air bubbles in the PCR reaction tube. Close the tube cap tightly.
- Do not use the reagents if the outer packaging of dry powder reagents is damaged or leaked.
- Do not use the reagents if desiccant color changes from orange to dark green.
- Do not use the reagents if the lyophilized reagent in MPXV PCR Reaction Tube is damaged or atrophied.

Reagent Storage and Handling

Reagent Storage

- All reagents should be stored at 2~30°C in a dry place and kept away from light before opening.
- The production date and expiration date are printed on the outer package.
- The storage method of the kit after opening is shown in the following table.

Type of reagent	***Before opening the foil pouch***	***After dissolution***
MPXV PCR Reaction Tube	Store at 2-30°C in a dry place and keep away from light.	Use immediately.
MPXV Positive Control		Use immediately and discard any remaining liquid.

Type of reagent	***Before opening tube***	***After opening tube***

MPXV Negative Control	Store at 2-30°C in a dry place and keep away from light.	Use immediately and discard any remaining liquid.
Molecular Grade Water		

Reagent Handling

- The MPXV Positive Control should be reconstituted with **400µL** Molecular Grade Water before use.
- Liquid reagents, MPXV Negative Control and Molecular Grade Water should be thoroughly mixed at room temperature before use.
- Do not use reagents from different batches interchangeably.

Specimen Collection, Transportation, and Storage

Collecting Specimens and Transporting Specimens

- The type of specimen is lesion swab (i.e., swab of human pustular or vesicular rash). [2]
- Lesion swab specimens are collected in viral transport media (VTM) or Universal Transport Medium (UTM).
- For specimen collection and transportation, please refer to the WHO document "Diagnostic testing for the monkeypox virus (MPXV)" [4].

Storing Specimens

- Specimens stored at 2-8°C can be tested up to 7 days from collection.
- Specimens stored frozen (-20°C or lower) can be tested up to 30 days from collection.
- The temperature for longer-term specimen storage (>30 days from collection) is recommended at -70°C [2].
- The specimen is allowed a maximum of one freeze-thaw cycle.

Test Procedure

1. Preparation

- Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. Decontamination agents should be used including 10% bleach, 75% ethanol, and DNAzap™ to minimize the risk of nucleic acid contamination.
- The MPXV Positive Control should be reconstituted with **400µL** Molecular Grade Water before use. Nucleic acid extraction is required for all controls (MPXV Positive Control and MPXV Negative Control).

2. Sample Extraction

There are three extraction methods verified by this Kit. Different extraction method corresponds to different extraction kit. Please select one of the following extraction methods for extraction.

It is noted that MPXV Positive Control and MPXV Negative Control in this kit should be extracted with the same protocol as for specimens.

2.1 Extraction method 1

Recommendation(s): A total of 100µL of specimen (lesion swab in VTM or UTM) is added to 100µL of MagNA Pure External Lysis Buffer. Specimen lysis and inactivation is conducted prior to loading samples onto the Roche MagNA Pure 96 System for extraction. Then using the MagNA Pure DNA and Viral NA Small Volume Kit to extract nucleic acid. The final elution volume is 50µL.

The extraction procedure should be performed according to the manufacturer's instructions (except as noted in recommendations above). The extracted nucleic acid can be directly added to the PCR reaction immediately or stored at -70°C.

2.2 Extraction method 2

Use the Liferiver® AutoEX36 E Extraction Kit to extract nucleic acid. The extraction procedure should be performed according to the manufacturer's instructions. The extracted nucleic acid can be directly added to the PCR reaction immediately or stored at -70°C.

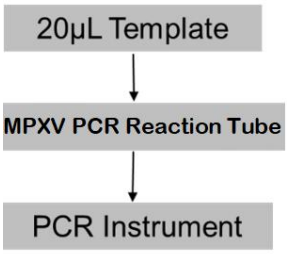
2.3 Extraction method 3

Use the Viral DNA/RNA Isolation Kit (Centrifuge Column) to extract nucleic acid. The extraction procedure should be performed according to the manufacturer's instructions. The extracted nucleic acid can be directly added to the PCR reaction immediately or stored at -70°C.

3. Template Addition

Note: Plate set-up configuration can vary with the number of specimens and work day organization. Negative control and positive control must be included in each run.

1) Take n tubes of MPXV PCR Reaction Tube (n as the number of samples and controls to be tested).
2) Add 20µL template (nucleic acid extracted from negative control, positive control and specimens) to different MPXV PCR Reaction Tube.



3) Dissolve the dry powder completely: Repeatedly pipette up and down, or cover the tube cap and oscillate to mix well. Centrifuge briefly.
4) Put the reaction tube into PCR instrument and carry out the amplification reaction immediately.

4. PCR Program Settings

For this kit, the reaction volume is 20µL. Perform the following protocol:

37°C for 2min	1cycle
95°C for 90sec	
95°C for 5sec, 63°C for 30sec (Fluorescence measured at 63°C)	42cycles

Selection of Fluorescence Channels	
FAM	MPXV
Texas Red	IC

Note: For Applied Biosystems 7500 (Software Version 2.3), please choose “none” as quencher and passive reference.

For more details on instrument settings, please refer to the “Appendix 1: Operation Procedure on ABI7500 (Software Version 2.3)” and “Appendix 2: Operation Procedure on Life9600 (Software Version LifeQP 1.2.1)” of this manual.

5. Data Analysis

5.1 Data analysis on Applied Biosystems 7500 Real-Time PCR System (Software Version 2.3)

1) Once the run has completed, select the **Analysis** tab at the upper left corner of the software.
2) Select the Amplification Plot tab to view the raw data (see **Figure 1**).
3) Start by highlighting all samples from the run; to do this, click on the upper left hand box **(a)** of the sample wells (see **Figure 1**). All the growth curves should appear on the graph.
4) On the top of the window **(b)**, the **Plot Type** drop down selection should be set to **Δ Rn vs Cycle**. The **Graph Type** drop down selection should be set to **Linear (c)**.
5) Select **1** from **(d)** the Target drop down menu, using the downward arrow. Please note that each target is analyzed individually to reflect different performance profiles of target.
6) Cancel the check of **Auto** in **Threshold** and **Auto Baseline**. **(e)**
7) Add the check of **Threshold** and **Baseline Start** in **Show (f)**.
8) Using the mouse, click and drag the blue threshold line **(g)** until it lies within the exponential phase of the fluorescence curves.
9) Baseline setting principle: Select the region with relatively stable fluorescence signal before exponential amplification; the starting point (Start) setting should avoid signal fluctuation area at the initial stage of fluorescence acquisition; the end point (End) should be set in the region that is lower than the Ct/Cq value of the earliest exponentially amplified sample for 1 or 2 cycles. **(h)**

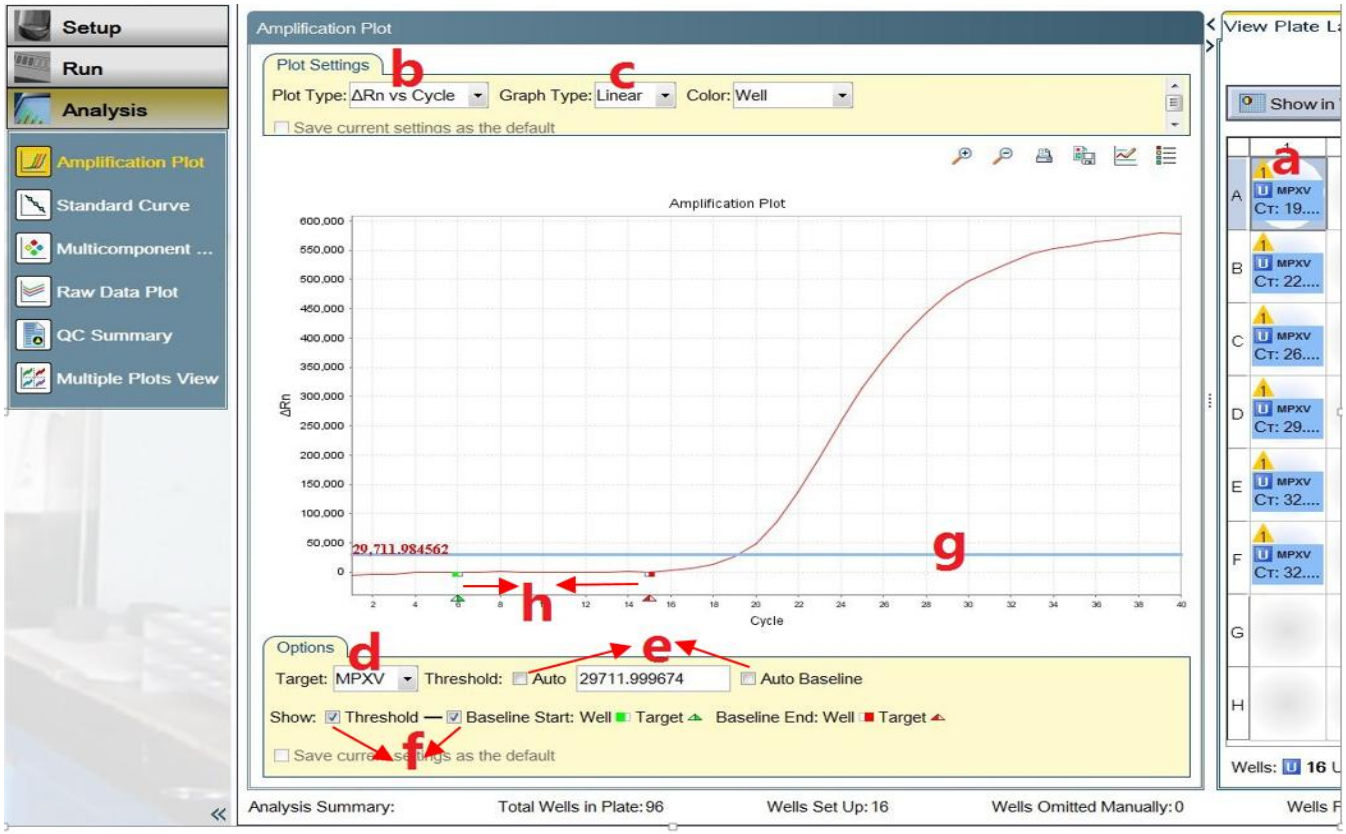


Figure 1. Amplification Plot Window on Applied Biosystems 7500 (Software Version 2.3)

10) Click the **Reanalyse** button at the upper right corner of the window.
11) Repeat Steps 5-9 to analyze results generated for each set of markers (i.e. 1, 2, 3, etc.).
12) Save analysis file by selecting **File** and then **Save As** from the main menu.
13) After completing analysis for each of the markers, click the **Export** tab, and then the **Export Data** screen (see **Figure 2**) will appear. Select **Customise Export** to display the Ct values (see **Figure 2**).
14) To filter report by sample name in ascending or descending order, simply click on **Sample Name** in the table.

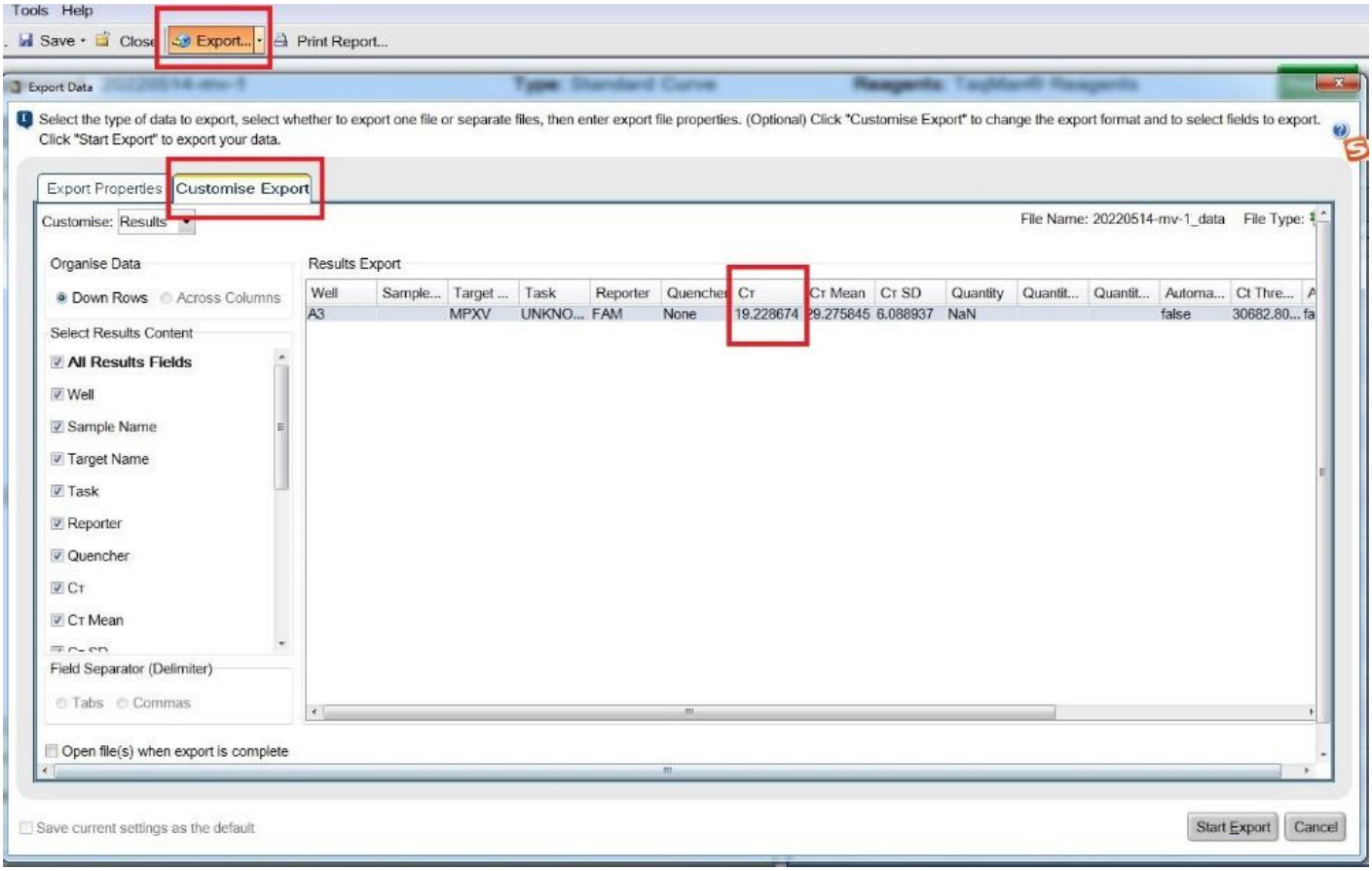


Figure 2. View Well Table on Applied Biosystems 7500 (Software Version 2.3)

5.2 Data Analysis on Life9600 Real-Time PCR System (Software Version LifeQP 1.2.1)

1) Once the run has completed, select the **Analysis** tab; the Experimental Analysis screen is used to view and analyze the experimental results.
2) Baseline and threshold lines generally adopt default values. If the baseline and threshold lines need to be adjusted, please refer to the PCR instrument manual or contact the manufacturer's technical support.
3) Click the Analyze button and choose the channel, and then select the well to read the Ct value (see **Figure 3**) on the upper left side and amplification curve on the lower right side. The amplification results of MPXV target gene are shown in the FAM channel. The test results of Internal Control are shown at the Texas Red channel.

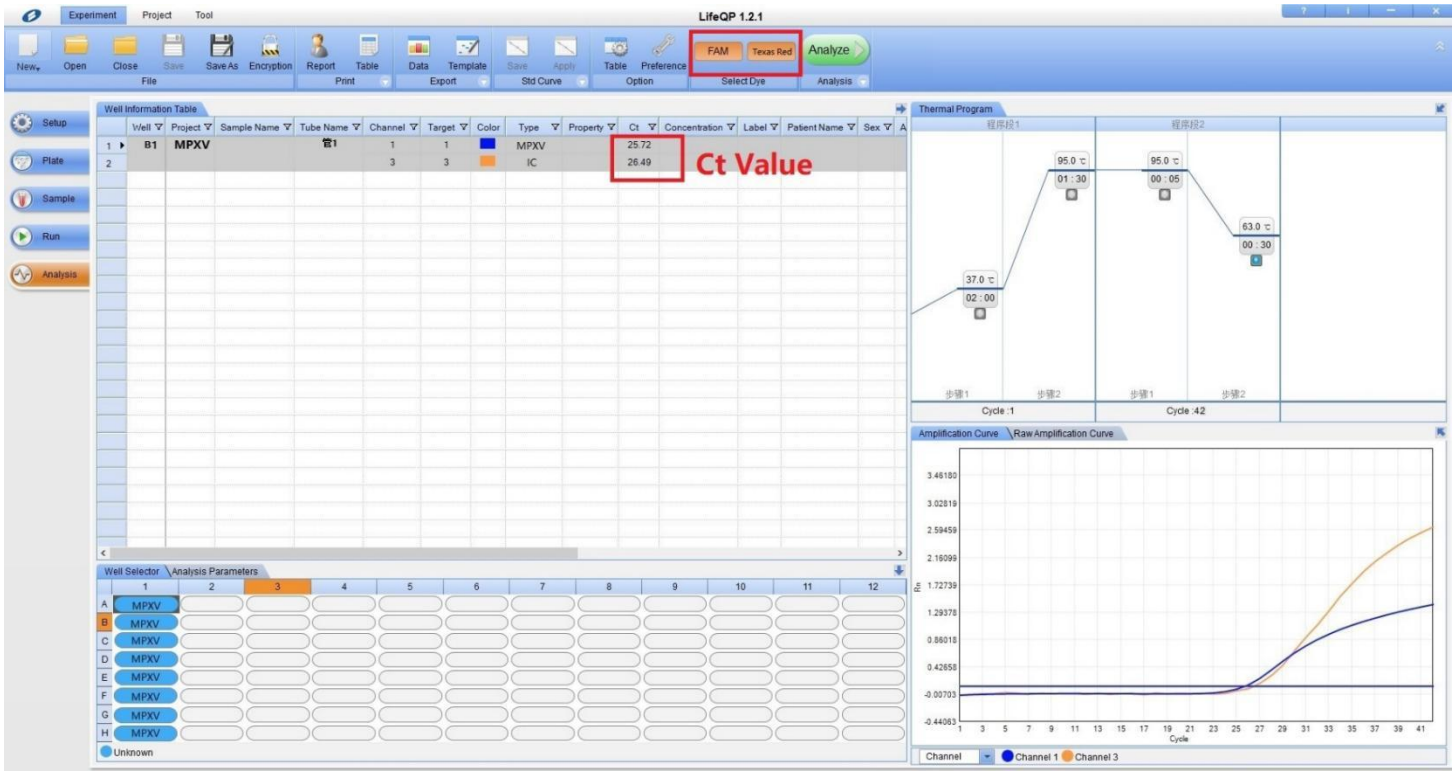


Figure 3. View Well Table on Life9600 (Software Version LifeQP 1.2.1)

Test Results

1. Quality Control Result Interpretation

Expected Performance of Controls Included in Monkeypox Virus Lyo-PCR Kit

Control Name	***Detection Target***	
	MPXV	***IC***
MPXV Positive Control[a]	Ct≤36	Valid amplification curve
MPXV Negative Control[b]	Undetermined	Undetermined

If any of the above controls does not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

Note:

a. Successful amplification (Ct value ≤ 36) of MPXV Positive Control indicates normal functionality of PCR reagents, instrument parameters, and operational procedures. If amplification fails, it suggests reagent degradation or operational errors. When no virus is detected in test samples, a normal positive control result excludes reagent-related issues.

b. If an amplification signal is detected in MPXV Negative Control, it indicates contamination in the experimental environment—such as reagent contamination, aerosol contamination, or cross-contamination between samples. In such cases, the experiment must be terminated immediately to identify and eliminate the contamination source.

2. Patient Specimen Result Interpretation

Interpretation of clinical specimen test results can only be conducted after the positive and negative controls have been examined and their results are determined to be valid. If the results of controls are invalid, results for clinical specimens cannot be interpreted.

Ct Value		*Result Interpretation*[a]
MPXV	**IC**[c]	
Undetermined	Ct≤35	Negative, monkeypox virus DNA NOT detected.
Ct≤42	No requirement[b]	Positive, monkeypox virus DNA detected.
Undetermined	Ct>35 or Undetermined	The result is invalid. Sample should be retested; if the result is still invalid, a new specimen should be obtained.

Note:

a. Laboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system[6].

b. For specimens that test positive in the monkeypox target channel, the detection result of internal control channel is irrelevant and can be ignored. A high monkeypox virus DNA load in the sample can lead to a reduced or absent IC signal.

c.The internal control uses RNase P, a human housekeeping gene present in all nucleated cells. Successful amplification of this gene indicates adequate collection of human cellular material. If the internal control shows abnormal results alongside undetected monkeypox target genes, it suggests either sampling failure or the presence of excessive inhibitors suppressing the PCR reaction.

Troubleshooting

Problems	*Possible Causes*	*Suggestions*
Negative control contamination	Contamination occurred during PCR preparation.	1)Make sure that work space and instruments are decontaminated at regular intervals. 2)Repeat the PCR with new reagents in replicates. 3)If possible, close the PCR tubes directly after addition of the sample to be tested.
	Contamination occurred during extraction.	1)Make sure that work space and instruments are decontaminated at regular intervals. 2)Repeat the DNA isolation and PCR of the sample to be tested using new reagents.
No signal with positive control	The selected fluorescence channel for PCR data analysis does not comply with the protocol.	For data analysis, select the right fluorescence channel for the analytical target.
	Incorrect programming of the temperature profile.	Compare the temperature profile with the protocol, and repeat the PCR.
	Incorrect configuration of the PCR.	Check your work steps and ensure the accuracy of the pipette, and then repeat the PCR.
	The storage conditions for one or more kit components did not comply with the instructions given in “Reagent Storage”.	Check the storage conditions and the expiration date (See the kit label) of the reagents and, if necessary, use a new kit.
	The kit has expired.	Check the storage conditions and the expiration date

		(See the kit label) of the reagents and, if necessary, use a new kit.
IC failure	The PCR was inhibited.	Make sure that you use the recommended isolation method and closely follow the manufacturer's instructions.
	DNA was lost during extraction.	Check if the isolation kit and instrument work well. Make sure that you use the recommended isolation method and closely follow the manufacturer's instructions.
	The fluorescence channel Texas Red is not or incorrectly selected.	Select the fluorescence channel Texas Red for the internal control PCR.
	The PCR parameters are not set correctly.	Compare the PCR parameters in the result file with that in the instructions for use.
	Incorrect configuration of the PCR setup.	Check your work steps and, if necessary, repeat the PCR.
	The storage conditions for one or more kit components do not comply with the information in the labels.	Check the storage conditions of the reagents and, if necessary, use a new kit.
	The Monkeypox Virus Lyo-PCR Kit has expired.	Check the storage conditions and the expiration date of the reagents and, if necessary, use a new kit.
Unexpected Ct shifts	Improper sample preservation leads to DNA degradation.	Check if the nucleic acid storage is appropriate, and if there are enough samples, re-extract the nucleic acid.
	The sample or detection reagents are insufficiently mixed, or the pipetting is uneven.	Check if the sample or detection reagents are thoroughly mixed, and ensure the pipettes are regularly calibrated.
	The PCR instrument was not calibrated, resulting in well differences.	Check if the PCR instrument is regularly calibrated, and contact technical support if necessary.
	Inadequate sealing of PCR reaction tubes leads to evaporation	After the PCR is completed, check whether the volume of liquid in the tubes has decreased.
Instrument error messages	Loose power cable, faulty power module, power supply unit failure, voltage fluctuation.	Check power connections and socket. Use a voltage stabilizer. Replace power module if necessary.
	Faulty sensor, calibration drift, blocked fan.	Restart instrument. Check/clean fans. Run temperature calibration. Replace sensor or module if faulty.
	Dirty optics, light source failure, detector issues, fluorescent dye problems.	Clean optical components (e.g., with lint-free swab). Check/replace lamp. Verify dye integrity and concentration.
	Improper plate sealing, reaction volume, plate positioning error.	Use recommended tubes/plates and seals. Ensure correct reaction volumes and plate seated correctly.
	Loose data cable, outdated firmware, software conflict, communication module failure.	Restart device & computer. Reconnect/update software/firmware. Check cable connections.

Note: These suggestions may be helpful in solving problems that may rise. For further questions or problems, please contact technical support at info@liferiverbiotech.com.

Limitations

- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.
- Performance of Monkeypox Virus Lyo-PCR Kit has only been established in lesion swab specimens (i.e., swabs of human pustular or vesicular rash).
- Negative results do not preclude monkeypox virus infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by monkeypox virus have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen. If the virus mutates in the PCR target region, monkeypox virus may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result.
- Performance of the test has only been established in lesion swabs collected in VTM/UTM. Performance of the test has not been evaluated for dry swabs or for lesion swabs collected in other transport media.
- The performance of this test has not been established for monitoring treatment of monkeypox virus infection.
- The performance of this test has not been established for screening of blood or blood products for the presence of monkeypox virus.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Due to the molecular revolution of viruses, there is an inherent risk for any PCR based test system that accumulation of mutations over time may lead to false negative results.
- Benzocaine Gel at ≥3 mg/mL can interfere with detection and cause invalid results, and should be avoided during specimen collection.
- A panel of 15 organisms (six viruses, six bacteria, two fungi, and human genomic DNA,See Table 7. Cross-reactivity studies) did not show false positive or false negative (interference).
- Samples with concentrations below the detection limit (<100 copies/mL) may fail to be detected, leading to false-negative results. For sample exceeding 10^{12} copies/mL, which surpasses the measurable Ct value range(0~42) of the reagent, may also produce false negative results.
- The kit is designed specifically for the detection of the monkeypox virus and does not cover other Orthopoxvirus species within its detection scope.
- The endogenous internal control can verify sample adequacy, monitor extraction efficiency, and detect PCR inhibition. However, in positive samples, excessive concentrations of monkeypox virus may interfere with the internal control, resulting in no detectable Ct value.
- Exceeding the specified storage conditions or freeze-thaw cycles may compromise the kit performance.
- Use of unvalidated specimen types, extraction methods, or amplification platforms may result in unreliable results.

Performance Characteristics

Analytical Sensitivity (Limit of Detection)

The Limit of Detection (LoD) of the assay was evaluated to determine the lowest concentration that was successfully detected with a probability of 95% or greater.

To determine the tentative LoD, the monkeypox virus (Clade IIb) was diluted to 1,600, 400, 100, 25, 6.25 copies/mL. Each dilution was extracted in 8 replicates with Liferiver® AutoEX36 E Extraction Kit, and then tested with 1 batch of Monkeypox Virus Lyo-PCR Kit on Applied Biosystems 7500 Real-Time PCR System (Software Version 2.3). The lowest concentration that gave 100% positive rate was defined as the tentative LoD. The positive rate of per concentration is shown in Table 1.

Table 1. The result (positive rate) of the tentative LoD

Concentration (copies/mL)	*Positive rate*
1,600	100%
400	100%
100	100%
25	62.5%
6.25	12.5%

To confirm the LoD, the monkeypox virus (Clade IIb) was diluted to 200, 100, 50 copies/mL. Twenty replicates per concentration were extracted with Liferiver® AutoEX36 E Extraction Kit, and then tested with 3 batches of Monkeypox Virus Lyo-PCR Kit on Applied Biosystems 7500 Real-Time PCR System (Software Version 2.3). The lowest concentration that gave at least 95% positive rate was defined as the final LoD. According to the result of Table 2, the final LoD of Monkeypox Virus Lyo-PCR Kit was 100 copies/mL.

Table 2. The result (positive rate) of the final LoD

Concentration (copies/mL)	*Positive rate*		
	Batch 1	*Batch 2*	*Batch 3*
200	100%	100%	100%
100	100%	100%	95%
50	70%	75%	75%

The LoD was then verified at a concentration of 100 copies/mL with 1 lot of Monkeypox Virus Lyo-PCR Kit by diluting monkeypox virus (Clade I and Clade IIb) across combinations of three extraction methods and two PCR instruments. The positive rate of each combination of extraction method and PCR instrument was 100% (20/20).

Inclusivity (*in silico* analysis)

The kit includes primers/probes that are specific for the F3L gene and B7R gene of monkeypox virus. The two genes were analyzed as follows:

F3L: An *in silico* inclusivity analysis was conducted by aligning the MPXV target primers and probe sequence against available monkeypox virus sequences as of September 23, 2024. The evaluation included a total of 6,340 monkeypox virus sequences, including 72 sequences of monkeypox virus (Clade Ia), 25 sequences of monkeypox virus (Clade Ib), 2 sequences of monkeypox virus (Clade IIa) and 6,241 sequences of monkeypox virus (Clade IIb). Of the 6,340 sequence entries for monkeypox virus included in the analysis, 6,338/6,340 (99.97%) sequences had 100% homology to MPXV target gene F3L, and 6,340/6,340 (100%) sequences had at least 90% match to MPXV primer/probes.

B7R: An *in silico* inclusivity analysis was conducted by aligning the MPXV target primers and probe sequence against available monkeypox virus sequences as of September 23, 2024. The evaluation included a total of 6,340 monkeypox virus complete genome sequences from GISAID, including 72 sequences of monkeypox virus (Clade Ia), 25 sequences of monkeypox virus (Clade Ib), 2 sequences of monkeypox virus (Clade IIa) and 6,241 sequences of monkeypox virus (Clade IIb). Of the 6,340 sequence included in the analysis, 6,339/6,340 (99.98%) sequences had 100% homology to MPXV target gene B7R, and 6,340/6,340 (100%) sequences had at least 90% match to MPXV primer/probes.

Cross-Reactivity (*in silico* analysis)

In Silico Cross-Reactivity Analysis of MPXV primers/probe against viral sequences

The kit includes primers/probes that are specific for the F3L gene and B7R gene of monkeypox virus. The two genes were analyzed as follows:

F3L: For the MPXV primer and probe sequences, 11 virus sequences were evaluated. Of the 1,760 viral sequences, 333 sequences matched both MPXV primers at least 80% (and possibly could result in amplicon), 347 sequences matched at least one MPXV primer more than 80% (and possibly could result in amplicon), 346 matched MPXV probe at least 80% (and possibly could result in a signal). The results are shown in Table 3.

The data indicate that the MPXV primer and probe may have cross-reactivity with Vaccinia virus, Cowpox Virus, Variola virus, Camelpox virus, Ectromelia virus and Buffalopox virus. But Cross-Reactivity Studies have shown that the kit has no cross-reaction with the six viruses above.

Table 3. Results of in silico cross-reactivity analysis of MPXV primer and probe vs viral sequences

Species	*Taxid*	*Upper level (genus)*	*Number of sequences evaluated*	*Sequences with ≥80% match to both primers*	*Sequences with ≥80% match to probe*	*Sequences with >80% match to at least one primer*
Vaccinia virus	10245	Orthopoxvirus	164	162	163	164
Cowpox Virus	10243	Orthopoxvirus	93	81	93	93
Variola virus (Smallpox)	10255	Orthopoxvirus	58	58	58	58
Camelpox virus	28873	Orthopoxvirus	10	10	10	10

Ectromelia virus	12643	Orthopoxvirus	13	13	13	13
Molluscum Contagiosum virus	10279	Molluscipoxvirus	31	0	0	0
Varicella-zoster virus (Chickenpox)	10335	Varicellovirus	269	0	0	0
Herpes simplex virus (HSV-1 and HSV-2)	10298 and 10310	Simplexvirus	757	0	0	0
Human papilloma virus (HPV)	10566	Papillomaviridae	318	0	0	0
Orf virus	10258	Parapoxvirus	38	0	0	0
Buffalopox virus	32605	Orthopoxvirus	9	9	9	9

B7R: For the MPXV primer and probe sequences, 11 virus sequences were evaluated. Of the 1,760 viral sequences, 346 sequences matched both MPXV primers at least 80% (and possibly could result in amplicon), 346 sequences matched at least one MPXV primer more than 80% (and possibly could result in amplicon), 279 matched MPXV probe at least 80% (and possibly could result in a signal). The results are shown in Table 4.

The data indicate that the MPXV primer and probe may have cross-reactivity with Vaccinia virus, Cowpox Virus, Variola virus, Camelpox virus, Ectromelia virus and Buffalopox virus. But Cross-Reactivity Studies have shown that the kit has no cross-reaction with the six viruses above.

Table 4. Results of *in silico* cross-reactivity analysis of MPXV primer and probe vs viral sequences

Species	***Taxid***	***Upper level (genus)***	***Number of sequences evaluated***	***Sequences with ≥80% match to both primers***	***Sequences with ≥80% match to probe***	***Sequences with >80% match to at least one primer***
Vaccinia virus	10245	Orthopoxvirus	164	164	164	164
Cowpox Virus	10243	Orthopoxvirus	93	92	92	92
Variola virus (Smallpox)	10255	Orthopoxvirus	58	58	4	58
Camelpox virus	28873	Orthopoxvirus	10	10	10	10
Ectromelia virus	12643	Orthopoxvirus	13	13	0	13
Molluscum Contagiosum virus	10279	Molluscipoxvirus	31	0	0	0
Varicella-zoster virus (Chickenpox)	10335	Varicellovirus	269	0	0	0
Herpes simplex virus (HSV-1 and HSV-2)	10298 and 10310	Simplexvirus	757	0	0	0
Human papilloma virus (HPV)	10566	Papillomaviridae	318	0	0	0
Orf virus	10258	Parapoxvirus	38	0	0	0
Buffalopox virus	32605	Orthopoxvirus	9	9	9	9

In Silico Cross-Reactivity Analysis of MPXV Primers/Probe Against Non-viral Sequences

The kit includes primers/probes that are specific for the F3L gene and B7R gene of monkeypox virus. The two genes were analyzed as follows:

F3L: The study conducted an *in silico* analysis for the MPXV primer/probe sequences against non-viral sequences from 23 microorganisms and Human Genomic DNA. Of the non-viral sequences evaluated, no sequence demonstrated ≥80% homology with both MPXV primers; there are 1,428 Staphylococcus aureus, 57 Human Genomic DNAs and >208 Escherichia coli that match ≥80% with MPXV probe but lack primer binding sites so the PCR amplification could not be completed; there are 311 Human Genomic DNA and >2000 Escherichia coli that match >80% with at least one primer, with the results shown in Table 5.

The data indicates that the MPXV primer and probe may have cross-reactivity with Staphylococcus aureus, Human Genomic DNA, Escherichia coli and Trichophyton rubrum. But Cross-Reactivity Studies have shown that the kit has no cross-reaction with the three pathogens and Human Genomic DNA above.

Table 5. Results of *in silico* cross-reactivity analysis of MPXV primer and probe vs non-viral sequences

Type	*Species*	*Taxid*	*Upper level (genus)*	*Sequences with ≥80% match to both primers*	*Sequences with ≥80% match to probe*	*Sequences with >80% match to at least one primer*
Bacteria	Staphylococcus aureus	1280	Staphylococcus	0	1,428	0
Bacteria	Streptococcus pyogenes	1314	Streptococcus	0	0	0
Bacteria	Pseudomonas aeruginosa	287	Pseudomonas	0	0	0
Bacteria	Corynebacterium jeikeium	38289	Corynebacterium	0	0	0
Human	Human Genomic DNA	9606	Homo	0	57	311
Bacteria	Escherichia coli	562	Escherichia	0	>208	>2000
Bacteria	Bacteroides fragilis	817	Bacteroides	0	0	0
Bacteria	Streptococcus Group G	1320	Streptococcus	0	0	0
Bacteria	Neisseria gonorrhoeae	485	Neisseria	0	0	0
Mycoplasma	Mycoplasma pneumoniae	2104	Mycoplasma	0	0	0
Spirochete	Treponema pallidum	160	Treponema	0	0	0
Bacteria	Streptococcus mitis	28037	Streptococcus	0	0	0
Parasite	Trichomonas vaginalis	5722	Trichomonas	0	0	0
Bacteria	Staphylococcus epidermidis	1282	Staphylococcus	0	0	0
Bacteria	Streptococcus agalactiae	1311	Streptococcus	0	0	0
Fungi	Trichophyton rubrum	5551	Trichophyton	0	0	1
Fungi	Candida albicans	5476	Candida	0	0	0
Bacteria	Lactobacillus species	1578	Lactobacillus	0	0	0
Bacteria	Corynebacterium diptheriae	1717	Corynebacterium	0	0	0
Trachomatis	Chlamydia trachomatis	813	Chlamydia	0	0	0
Mycoplasma	Mycoplasma genitalium	2097	Mycoplasma	0	0	0
Bacteria	Acinetobacter calcoaceticus	471	Acinetobacter	0	0	0

Bacteria	Enterococcus faecalis	1351	Enterococcus	0	0	0
Bacteria	Streptococcus Group C	1301	Streptococcus	0	0	0

B7R: The study conducted an *in silico* analysis for the MPXV primer/probe sequences against non-viral sequences from 23 microorganisms and Human Genomic DNA. Of the non-viral sequences evaluated, no sequence demonstrated ≥80% homology with both MPXV primers; there are >208 Escherichia coli that match ≥80% with MPXV probe but lack primer binding sites so the PCR amplification could not be completed; there are 23 Streptococcus pyogenes, 355 Human Genomic DNA, >2000 Escherichia coli, 320 Staphylococcus epidermidis, 31 Streptococcus agalactiae, 9 Candida albicans and 39 Streptococcus Group C that match >80% with at least one primer, with the results shown in Table 6.

The data indicate that the MPXV primer and probe may have cross-reactivity with Streptococcus pyogenes, Human Genomic DNA, Escherichia coli, Staphylococcus epidermidis, Streptococcus agalactiae, Candida albicans and Streptococcus Group C. But Cross-Reactivity Studies have shown that the kit has no cross-reaction with the six pathogens and Human Genomic DNA above.

Table 6. Results of in silico cross-reactivity analysis of MPXV primer and probe vs non-viral sequences

Type	*Species*	*Taxid*	*Upper level (genus)*	*Sequences with ≥80% match to both primers*	*Sequences with ≥80% match to probe*	*Sequences with >80% match to at least one primer*
Bacteria	Staphylococcus aureus	1280	Staphylococcus	0	0	0
Bacteria	Streptococcus pyogenes	1314	Streptococcus	0	0	23
Bacteria	Pseudomonas aeruginosa	287	Pseudomonas	0	0	0
Bacteria	Corynebacterium jeikeium	38289	Corynebacterium	0	0	0
Human	Human Genomic DNA	9606	Homo	0	0	355
Bacteria	Escherichia coli	562	Escherichia	0	>208	>2000
Bacteria	Bacteroides fragilis	817	Bacteroides	0	0	0
Bacteria	Streptococcus Group G	1320	Streptococcus	0	0	0
Bacteria	Neisseria gonorrhoeae	485	Neisseria	0	0	0
Mycoplasma	Mycoplasma pneumoniae	2104	Mycoplasma	0	0	0
Bacteria	Treponema pallidum	160	Treponema	0	0	0
Bacteria	Streptococcus mitis	28037	Streptococcus	0	0	0
Parasite	Trichomonas vaginalis	5722	Trichomonas	0	0	0
Bacteria	Staphylococcus epidermidis	1282	Staphylococcus	0	0	320
Bacteria	Streptococcus agalactiae	1311	Streptococcus	0	0	31
Fungi	Trichophyton rubrum	5551	Trichophyton	0	0	0
Fungi	Candida albicans	5476	Candida	0	0	9
Bacteria	Lactobacillus species	1578	Lactobacillus	0	0	0
Bacteria	Corynebacterium diptheriae	1717	Corynebacterium	0	0	0
Trachomatis	Chlamydia trachomatis	813	Chlamydia	0	0	0
Mycoplasma	Mycoplasma genitalium	2097	Mycoplasma	0	0	0
Bacteria	Acinetobacter calcoaceticus	471	Acinetobacter	0	0	0
Bacteria	Enterococcus faecalis	1351	Enterococcus	0	0	0
Bacteria	Streptococcus Group C	1301	Streptococcus	0	0	39

Cross-Reactivity Studies

The cross-reactivity studies were performed by testing potential cross-reactivity with a panel of 15 organisms (six viruses, six bacteria, two fungi, and human genomic DNA) whose sequences demonstrated ≥80% homology with MPXV primers or probes.

Each organism was tested in three replicates. There were no false positive results tested.

Table 7. Cross-reactivity studies

Organisms	*Concentration tested*	*Result*
Variola virus (Smallpox)	1E+05 copies/mL	Negative
Vaccinia virus	1E+05 PFU/mL	Negative
Cowpox virus	1E+05 copies/mL	Negative
Ectromelia virus	1E+05 copies/mL	Negative
Camelpox virus	1E+05 copies/mL	Negative
Buffalopox virus	1E+05 copies/mL	Negative
Staphylococcus aureus	1E+06 CFU/mL	Negative
Human Genomic DNA	1E+07 copies/mL	Negative
Staphylococcus epidermidis	1E+06 CFU/mL	Negative
Streptococcus agalactiae	1E+06 CFU/mL	Negative
Streptococcus pyogenes	1E+06 CFU/mL	Negative
Candida albicans	1E+06 CFU/mL	Negative
Trichophyton rubrum	1E+06 CFU/mL	Negative
Escherichia coli	1E+06 CFU/mL	Negative
Streptococcus Group C	1E+05 copies/mL	Negative

Microbial Interference Studies

The Monkeypox Virus Lyo-PCR Kit was evaluated for potential interference with a panel of 15 organisms (six viruses, six bacteria, two fungi, and human genomic DNA) whose sequences demonstrated ≥80% homology with MPXV primers or probes.

Low concentration of monkeypox virus was tested in the presence or absence of each organism. Three replicates were tested with Monkeypox Virus Lyo-PCR Kit for each organism. No interference was shown in the monkeypox virus with these organisms. The tested organisms and their concentrations are shown in Table 8.

Table 8. Microbial interference studies

Organisms	*Concentration tested*	*Interference observed*
Variola virus (Smallpox)	1E+05 copies/mL	None
Vaccinia virus	1E+05 PFU/mL	None
Cowpox virus	1E+05 copies/mL	None
Ectromelia virus	1E+05 copies/mL	None

Camelpox virus	1E+05 copies/mL	None
Buffalopox virus	1E+05 copies/mL	None
Staphylococcus aureus	1E+06 CFU/mL	None
Human Genomic DNA	1E+07 copies/mL	None
Staphylococcus epidermidis	1E+06 CFU/mL	None
Streptococcus agalactiae	1E+06 CFU/mL	None
Streptococcus pyogenes	1E+06 CFU/mL	None
Candida albicans	1E+06 CFU/mL	None
Trichophyton rubrum	1E+06 CFU/mL	None
Escherichia coli	1E+06 CFU/mL	None
Streptococcus Group C	1E+05 copies/mL	None

Interfering Substances

Twenty potential interfering substances (endogenous and exogenous) were assessed. Testing was performed with each potential interfering organism in low positive specimen (~3×LoD) and negative specimen. Three replicates were tested with Monkeypox Virus Lyo-PCR Kit for each interfering substance. The tested substances and their concentrations are shown in Table 9.

Of the 20 substances tested, Compound Benzocaine Gel at concentrations ≥3 mg/mL was observed to cause interference. The remaining interfering substances did not show false negative or false positive results.

Table 9. Interference studies

Interfering substances	*Concentration tested*	*Interference observed*
Acyclovir	7 mg/mL	None
Albumin	2.2 mg/mL	None
Benadryl cream/ointment	7 %	None
Blood/EDTA	5 %	None
Casein	7 mg/mL	None
Cornstarch	2.5 mg/mL	None
Douche	7 %	None
Feces	0.22 %	None
Female urine	10 %	None
Hydrocortisone cream	7 %	None
Lubricant	7 %	None
Male urine	10 %	None
Mucin	60 µg/mL	None
Neosporin	3 %, 5 %, 7 %	None
Seminal fluid	7 %	None
Zinc Oxide ointment	7 %	None
Compound Benzocaine Gel	1 mg/mL, 3 mg/mL, 5 mg/mL	≥ 3mg/mL
Docosanol containing cold sore	7 %	None

treatment		
Lidocaine cream	7 %	None
Petrolatum lip care	7 %	None

Clinical Performance Evaluation

Testing of natural clinical lesion swab specimens in VTM or UTM:

The performance of Monkeypox Virus Lyo-PCR Kit was evaluated by testing leftover lesion swab specimens in either universal transport medium (UTM) or viral transport medium (VTM) collected between June 2023 to October 2023 from patients suspected of monkeypox infection in USA.

In total, 70 de-identified natural clinical swab specimens were enrolled, of which 30 positive specimens including 10 low positive specimens (10/30, 33.3%) and 38 negative specimens were included in the evaluation. Two specimens were excluded due to insufficient specimen volume for completing all tests. At end, 68 specimens were analyzed for monkeypox virus detection with both Monkeypox Virus Lyo-PCR Kit and a comparator FDA-cleared molecular test in a blinded study.

The results are summarized in Table 10. The positive percent agreement (PPA) between Monkeypox Virus Lyo-PCR Kit and the comparator test was 100% (N = 30/30); the negative percent agreement (NPA) between Monkeypox Virus Lyo-PCR Kit and the comparator test was 100% (N = 38/38).

Table 10. Clinical performance results using lesion swab specimens

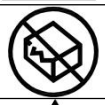
		FDA cleared real-time PCR assay		
		Positive	***Negative***	***Total***
Monkeypox Virus Lyo-PCR Kit	***Positive***	30	0	30
	Negative	0	38	38
	Total	30	38	68
PPA		100.00% (CI: 88.65%~100.00%)		
NPA		100.00% (CI: 90.82%~100.00%)		

References

1. World Health Organization. Target product profiles for tests used for mpox (monkeypox) diagnosis. 2023 Jul; Available from: https://apps.who.int/iris/handle/10665/371297
2. Testing Patients for Monkeypox. https://www.cdc.gov/poxvirus/monkeypox/clinicians/prep-collection-specimens.html
3. Sklenovska N, Van Ranst M. Emergence of M as the most important orthopoxvirus infection in humans[J]. Frontiers in public health, 2018, 6: 241.
4. Diagnostic testing for the monkeypox virus (MPXV) Interim guidance.WHO, 10 May 2024.

5. World Health Organzation. Clinical management and infection prevention and control for monkeypox: Interim rapid response guidance. 2022 Jun 10; Available from: https://www.who.int/publications/i/item/WHO-MPX-Clinical-and-IPC-2022.1
6. US Food and Drug Administration. Monkeypox (mpox) Emergency Use Authorizations for Medical Devices. 2023 May 24; Available from: https://www.fda.gov/medical-devices/emergency-use-authorizations-medical-devices/monkeypox-mpox-emergency-use-authorizations-medical-devices.

Symbols

	**In vitro diagnostic medical device**		**Date of manufacture**
	**Use by date**		**Consult Instructions for Use**
	**Temperature limit**		**Manufacturer**
	**Batch code**		**Catalogue number**
	**Contains sufficient for <n> tests**		**Do not use if package is damaged**
	**Keep away from sunlight**		**Non-sterile**

Contact Information, Ordering, and Product Support

For technical and product support, contact the manufacturer directly.

Users should immediately notify manufacturer and local regulatory authority in the event of a serious incident, such as repeated assay failure, an unexpected increase in invalid results, or other suspected public health impacts.

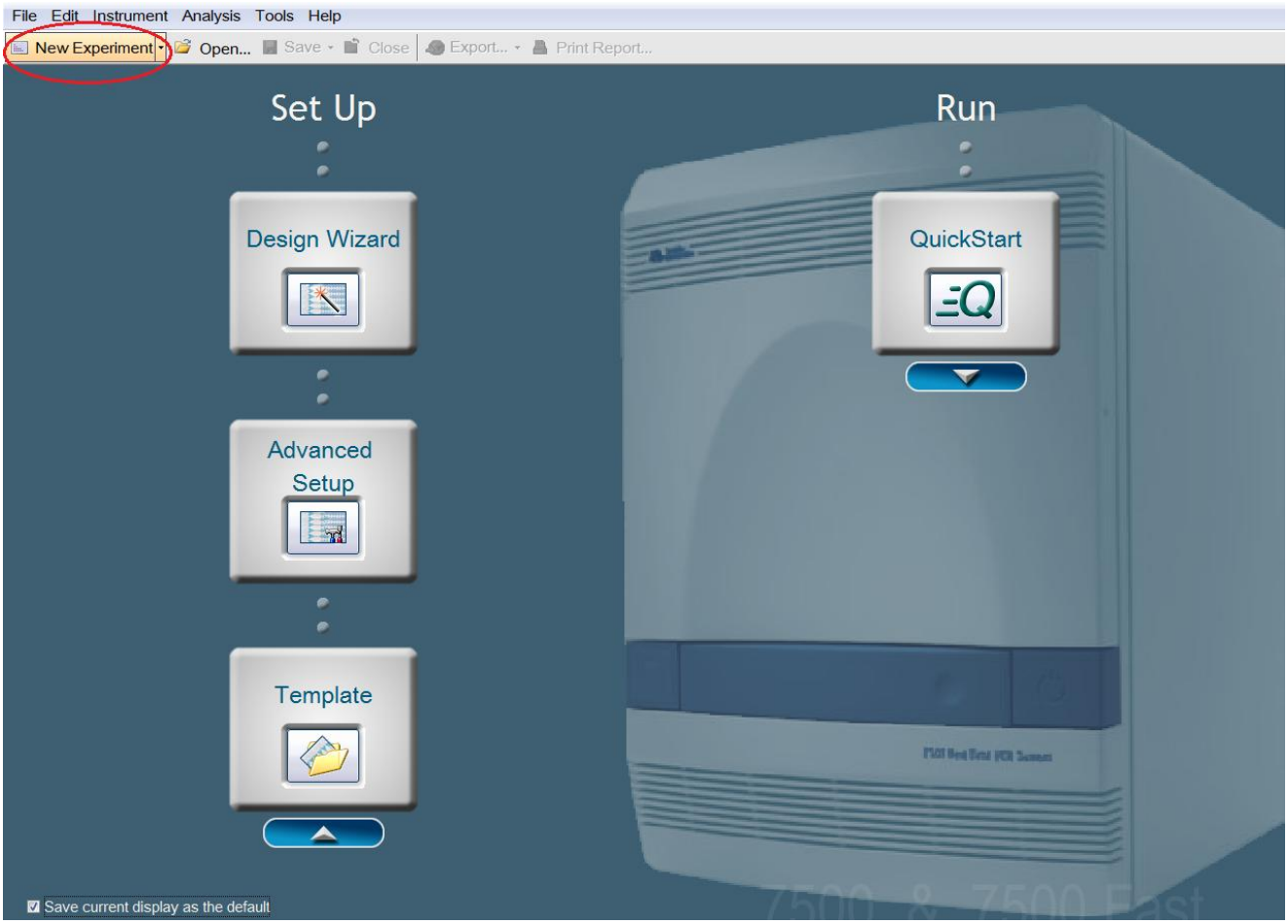
Send email to: info@liferiverbiotech.com

Or Fax to: +86-21-34680595

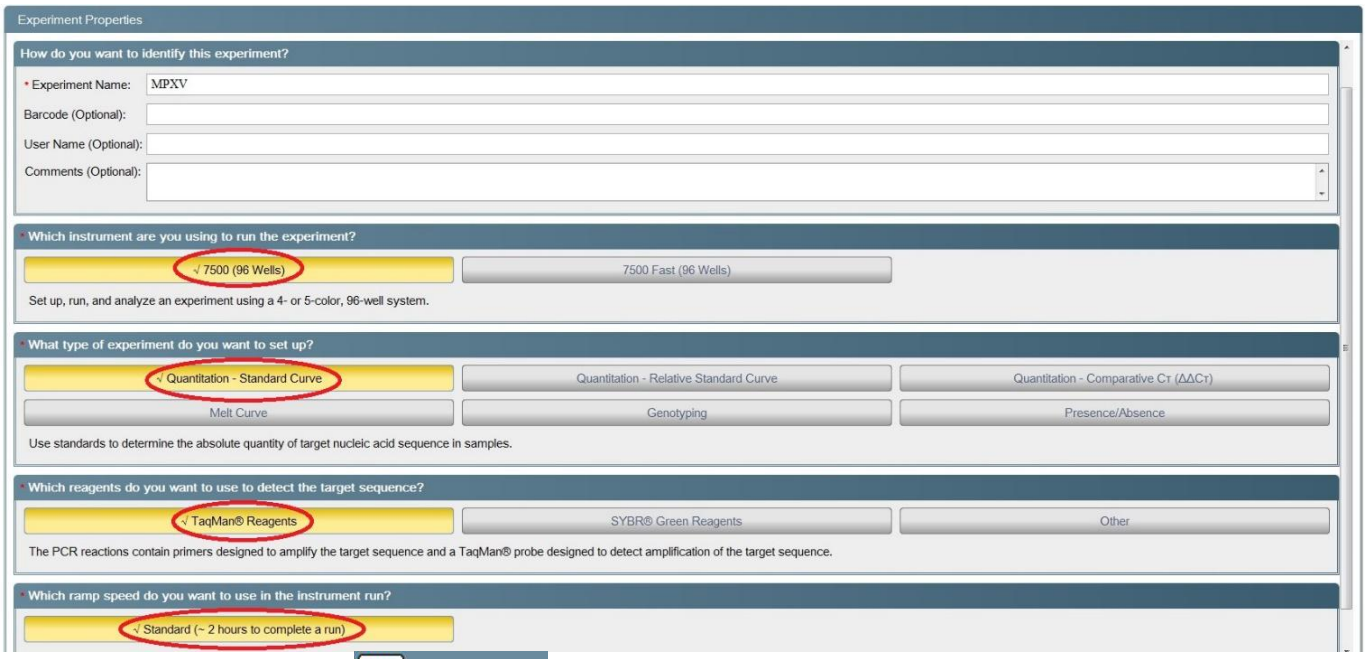
Or Tel to: +86-21-34680598

Appendix 1: Operation Procedure on ABI 7500 (Software Version 2.3)

1. Double click on the ABI system icon on the desktop to enter the program (Software version: v2.3).
2. Click “New Experiment” from the quick start up menu.

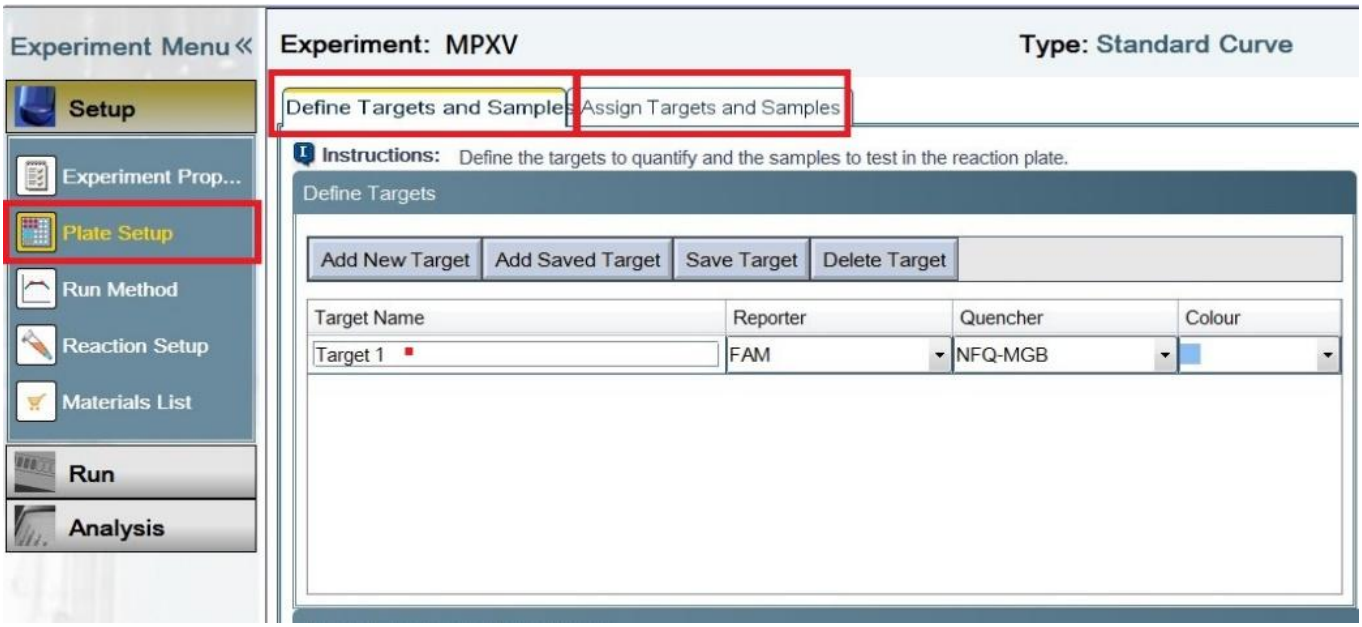


3. Enter the experiment name and choose the following items (Usually they are set as defaults).

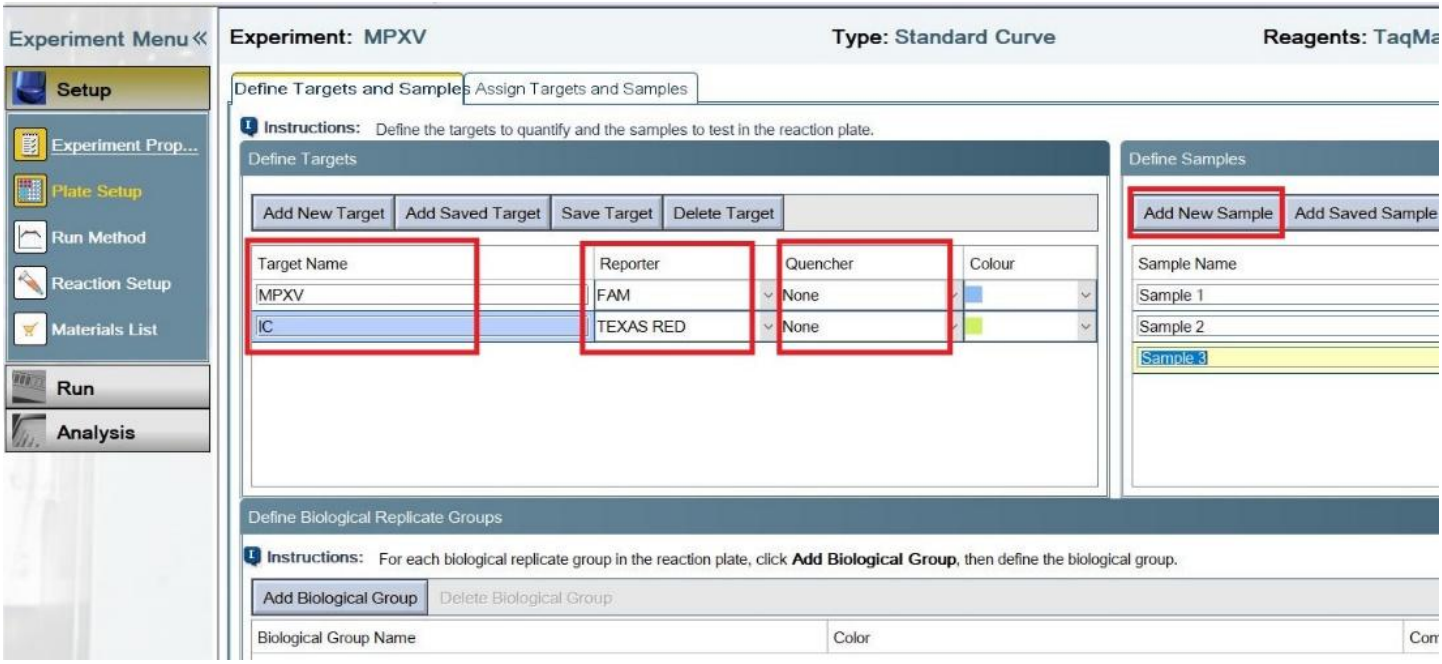


4. Click Plate Setup button 

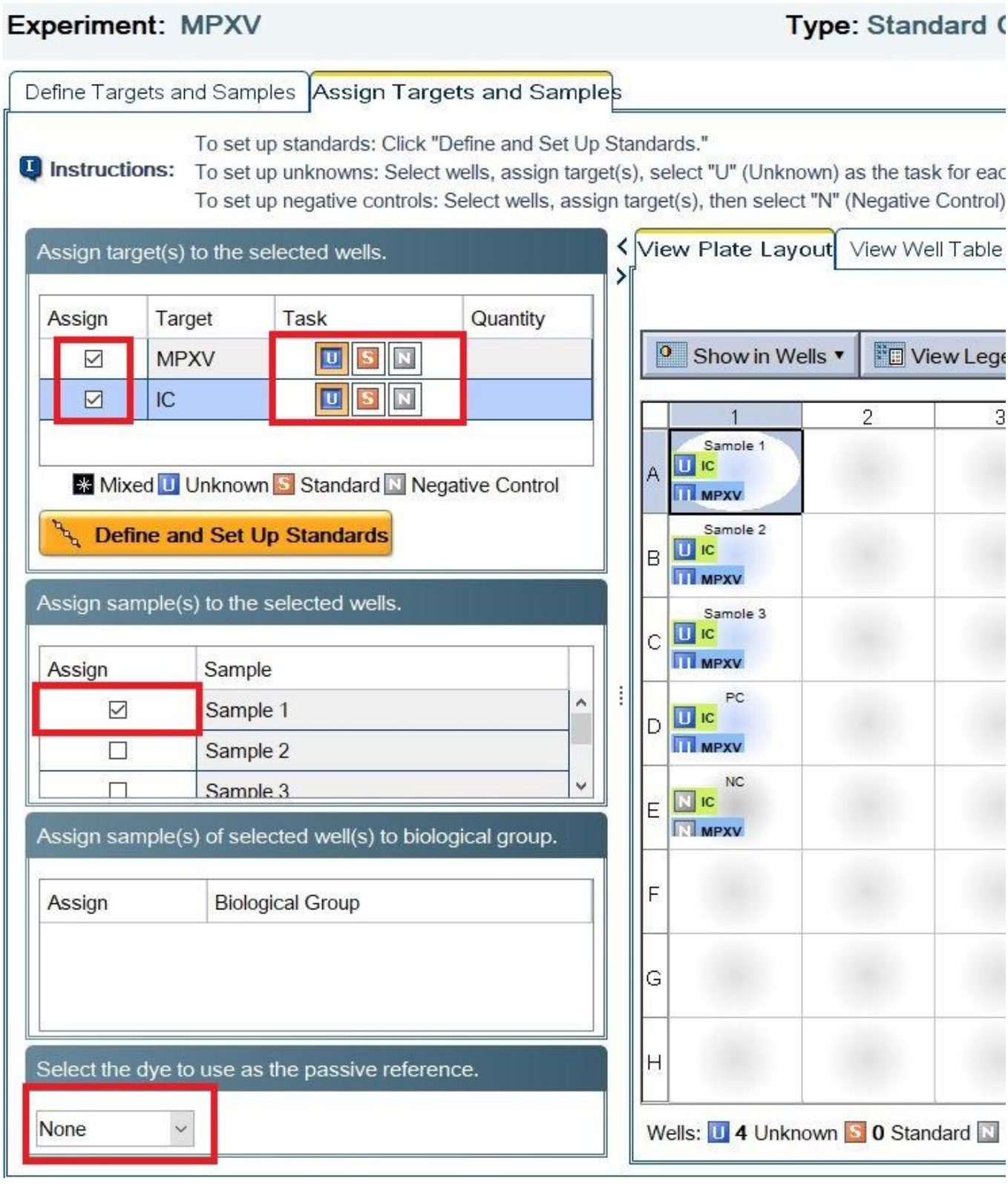
. Then “Define Targets and Samples” and “Assign Targets and Samples” will appear as below.



5. Click “Define Targets and Samples” to set up sample information. Under “Define Targets”, click “Add New Target”. Enter the target name (MPXV &IC) and select the corresponding reporters (FAM & TEXAS RED). Under “Quencher”, choose “None”. Under “Define Samples”, add new samples and enter the sample names.



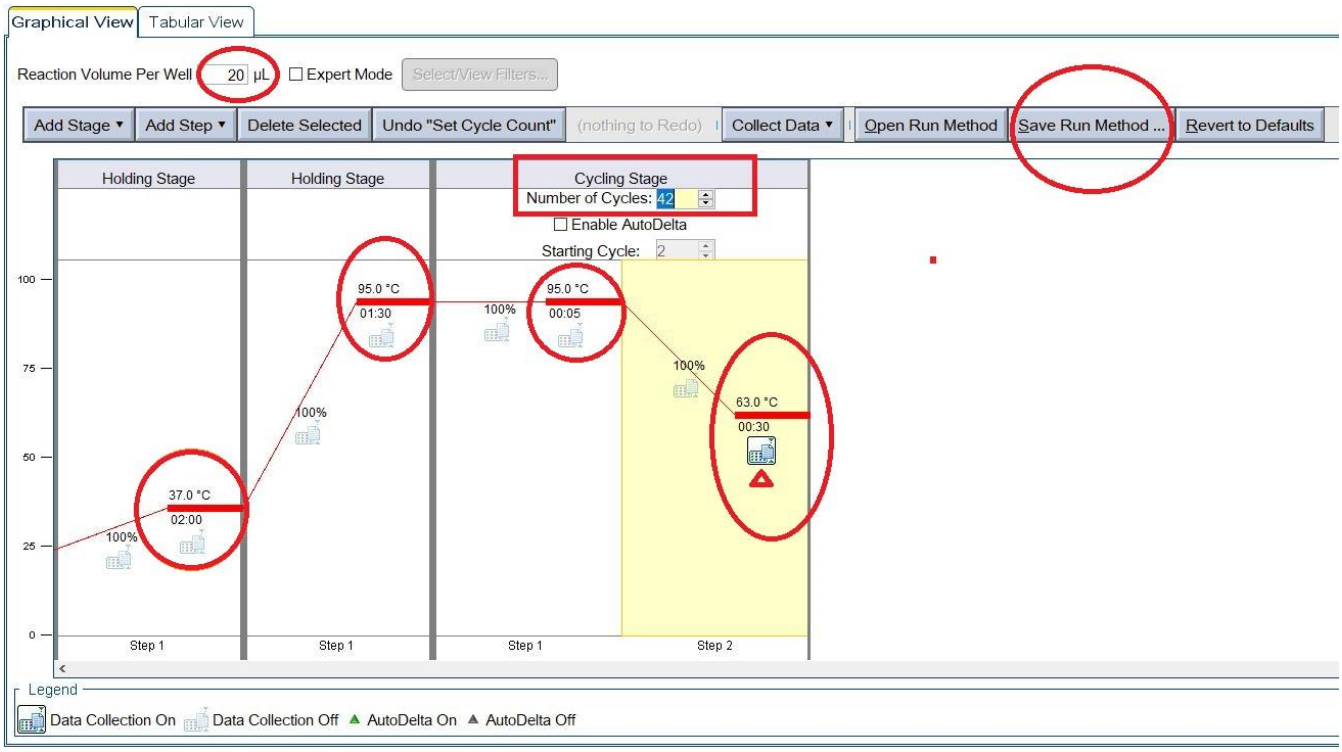
6. Click “Assign Targets and Samples” to layout samples. Click to choose reaction wells. Then tick the samples on the left and specify the reaction well under “Task” tab (S means a standard, while U represents an unknown sample and N is a negative control; there is no positive in the task column, so you may edit a PC name to assign the positive control). Meanwhile, tick FAM&VIC. Then choose “None” under “Select the dye to use as the passive reference”.



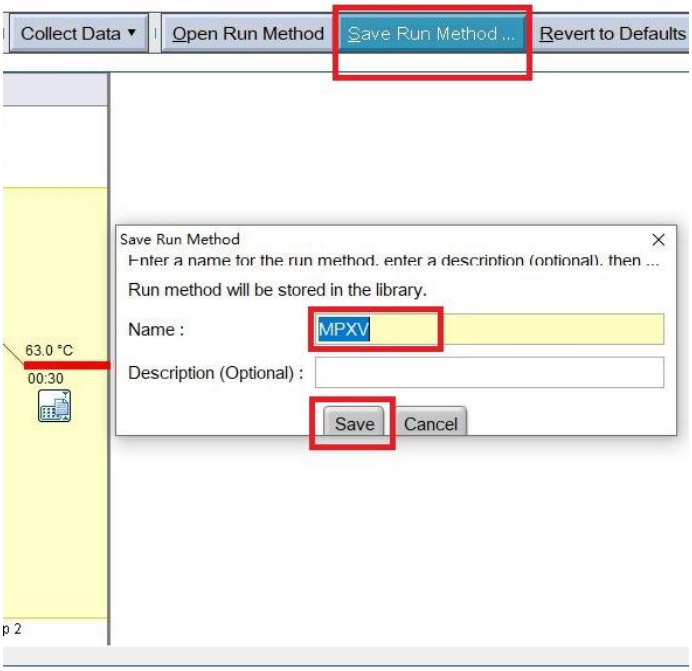
7. Click “Run Method” on the left side.

8. Set the parameters as follows:

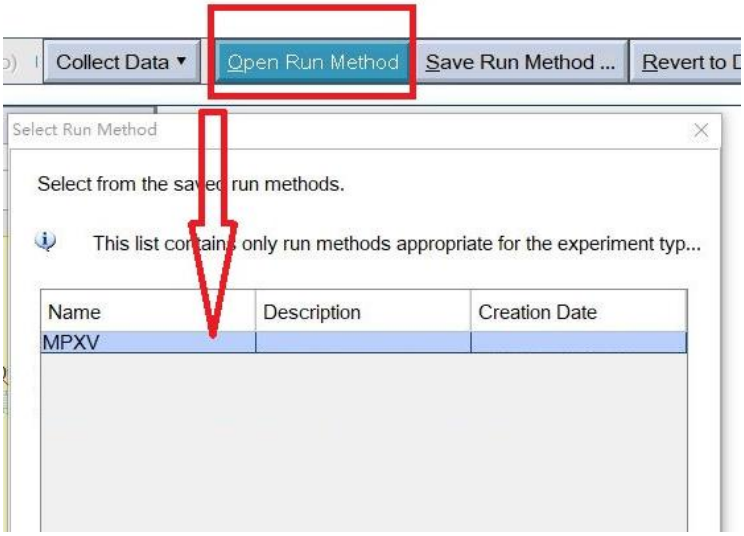
37°C for 2min	1cycle
95°C for 90sec	
95°C for 5sec, 63°C for 30sec (Fluorescence measured at 63°C)	42cycles



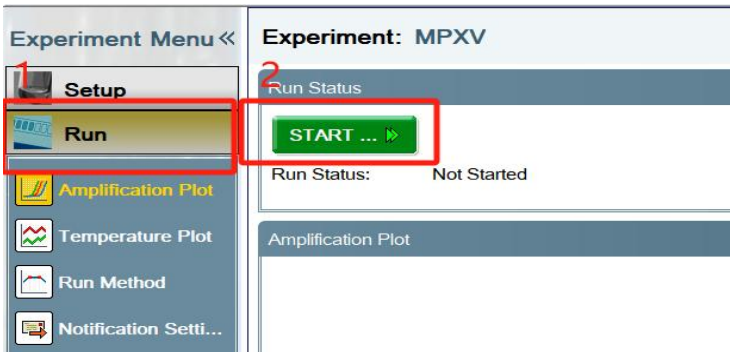
Here, you can save the set program: Click “Save Run Method”, name the program and save it.

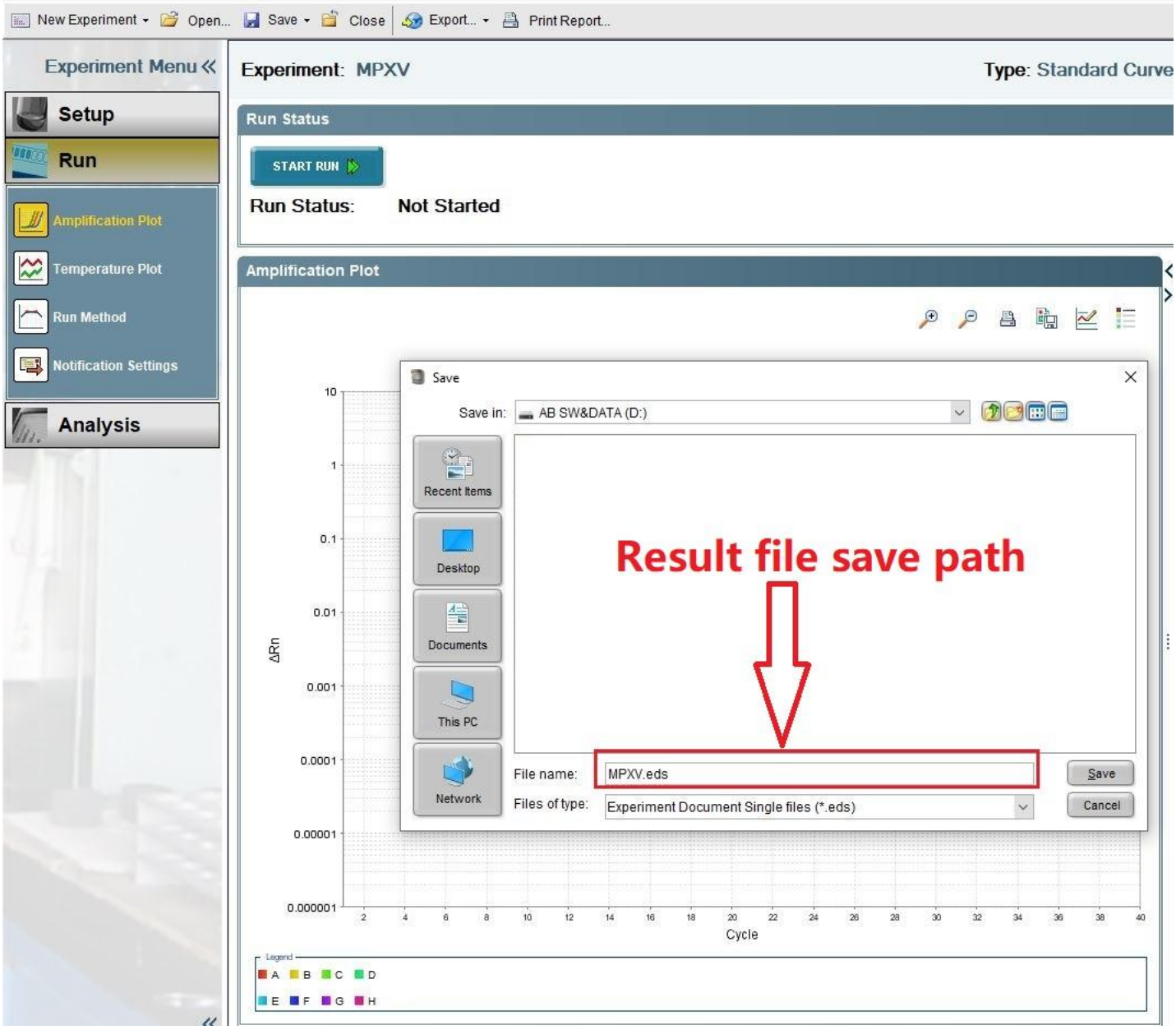


The program can be directly opened and used for next experiment.



9. Click **Run** and then Click the button  and, in the pop-up interface, enter the save path to save the document. Then click “Save” to run the test.


Experiment Menu
Experiment: MPXV
1
2
Setup
Run
Run Status
START ...
Run Status: Not Started
Amplification Plot
Temperature Plot
Run Method
Notification Setti...
Amplification Plot

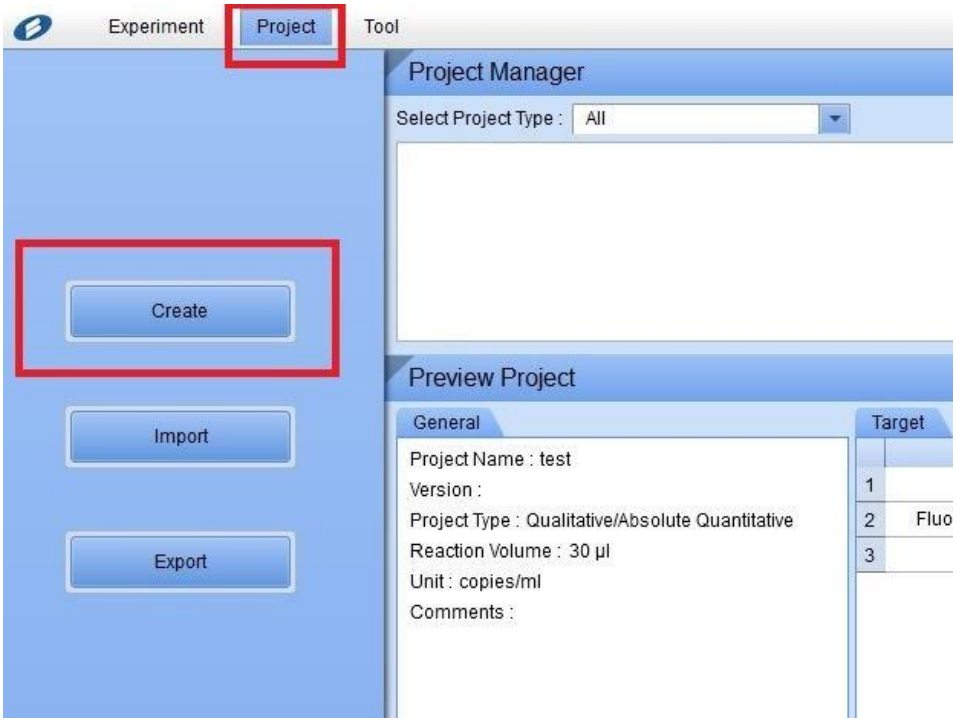

New Experiment
Open...
Save
Close
Export...
Print Report...
Experiment Menu
Experiment: MPXV
Type: Standard Curve
Setup
Run
Run Status
START RUN
Run Status: Not Started
Amplification Plot
Temperature Plot
Run Method
Notification Settings
Analysis
Amplification Plot
Save
Save in: AB SW&DATA (D:)
Recent Items
Desktop
Documents
This PC
Network
Result file save path
File name: MPXV.eds
Files of type: Experiment Document Single files (*.eds)
Save
Cancel
ΔRn
Cycle
Legend
A B C D
E F G H

Appendix 2： Operation Procedure on Life9600 (Software Version LifeQP 1.2.1)

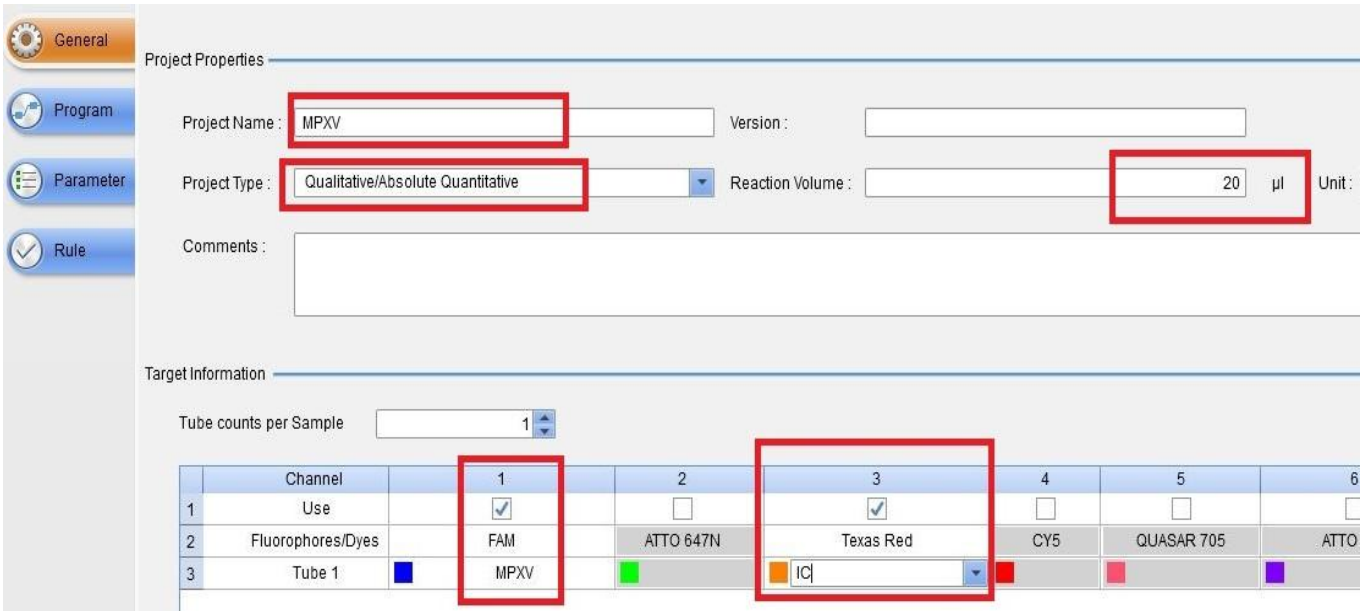
1. Enter the Life9600 PCR program by double clicking on the system icon 

on the desktop (Software version LifeQP 1.2.1).

2. Click the button“Project” to enter the following interface and then click "Create" to create a new program;

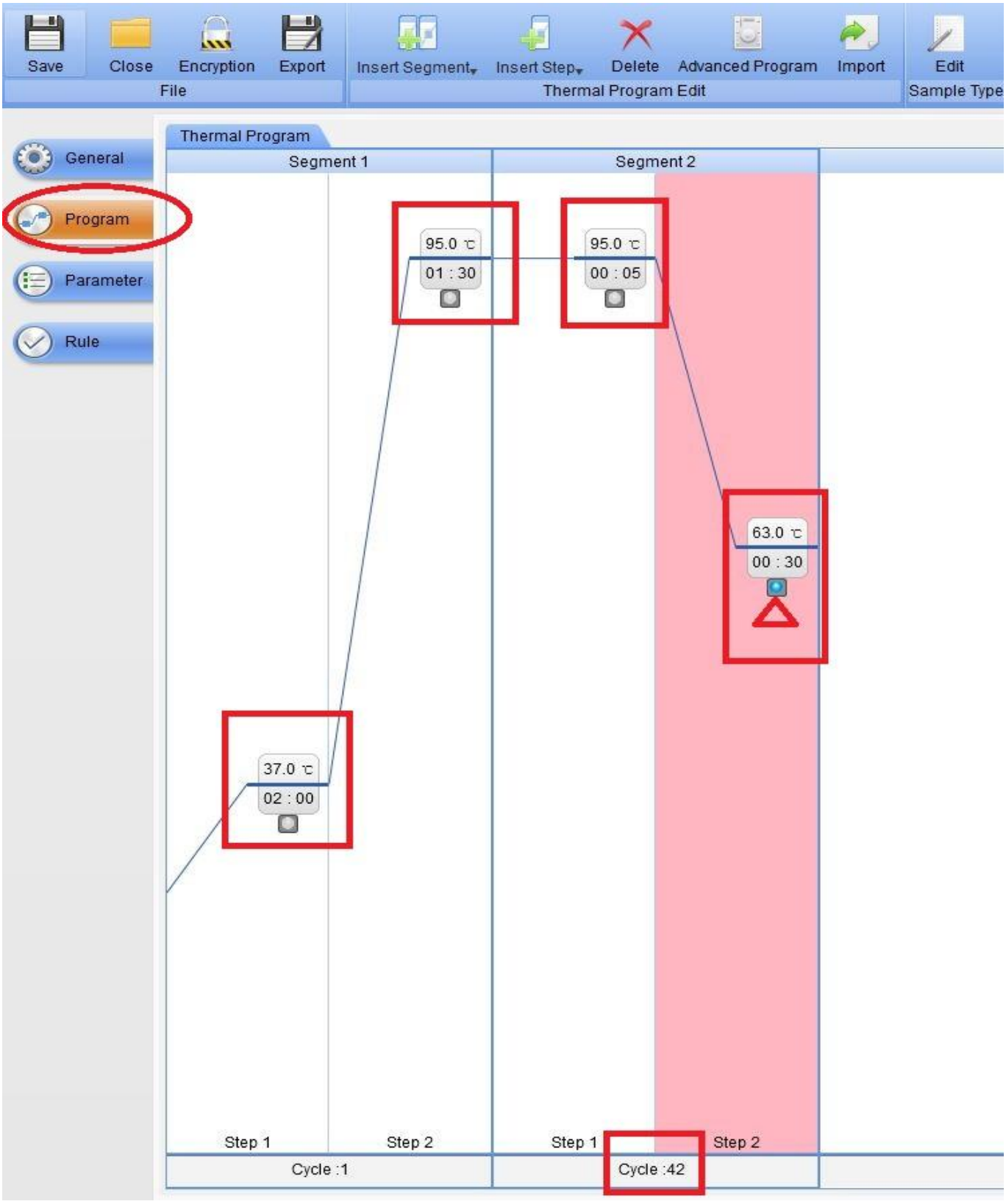


3. The below window will appear. Enter Project Name, Project Type and Reaction Volume, tick the channel (FAM & Texas Red) and enter the target name (MPXV & IC).

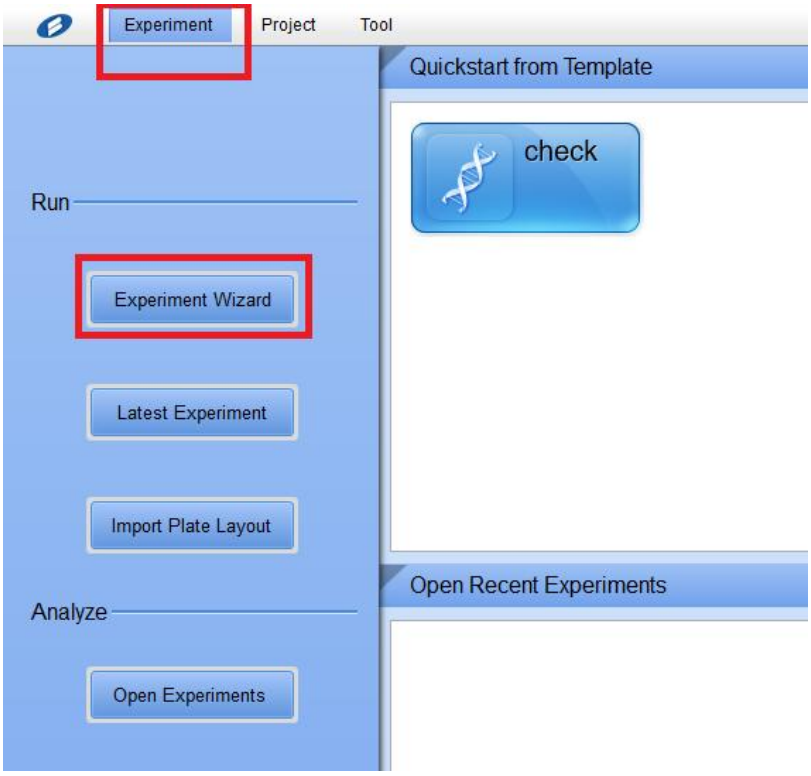


4. Click the button “Program” to enter the following interface. Set parameters in the new window, and click the "Save" button in the upper corner to save the parameters. **The edited parameters will appear in the “Select Project File” in step 7 and can be directly used for next experiment (Next experiment can start directly from step 6).** Click “Close” to exit.

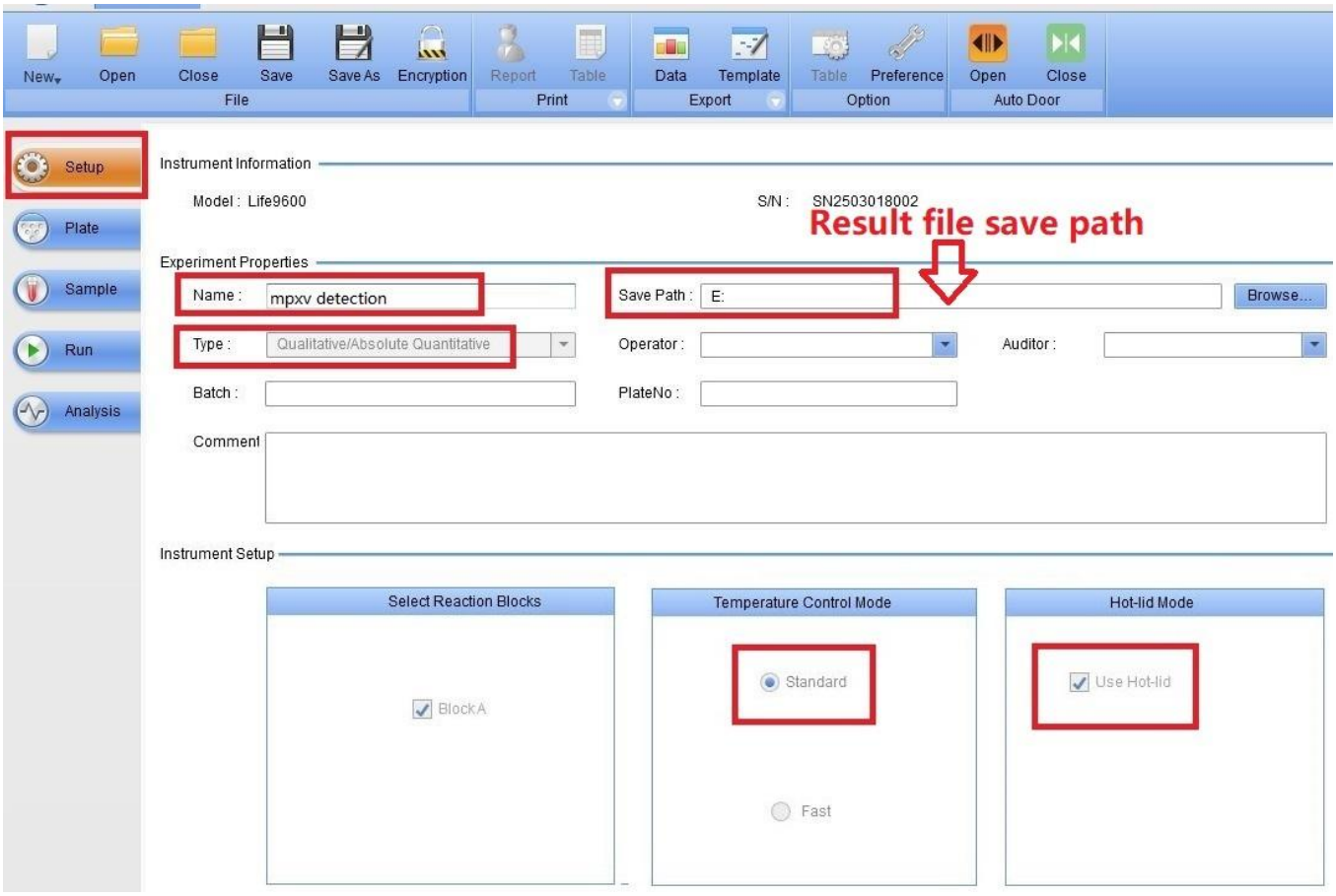
37°C for 2min	1cycle
95°C for 90sec	
95°C for 5sec, 63°C for 30sec (Fluorescence measured at 63°C)	42cycles


Save
Close
Encryption
Export
File
Insert Segment
Insert Step
Delete
Advanced Program
Import
Thermal Program Edit
Edit
Sample Type
General
Program
Parameter
Rule
Thermal Program
Segment 1
Segment 2
95.0 ℃
01 : 30
95.0 ℃
00 : 05
63.0 ℃
00 : 30
37.0 ℃
02 : 00
Step 1
Step 2
Step 1
Step 2
Cycle :1
Cycle :42

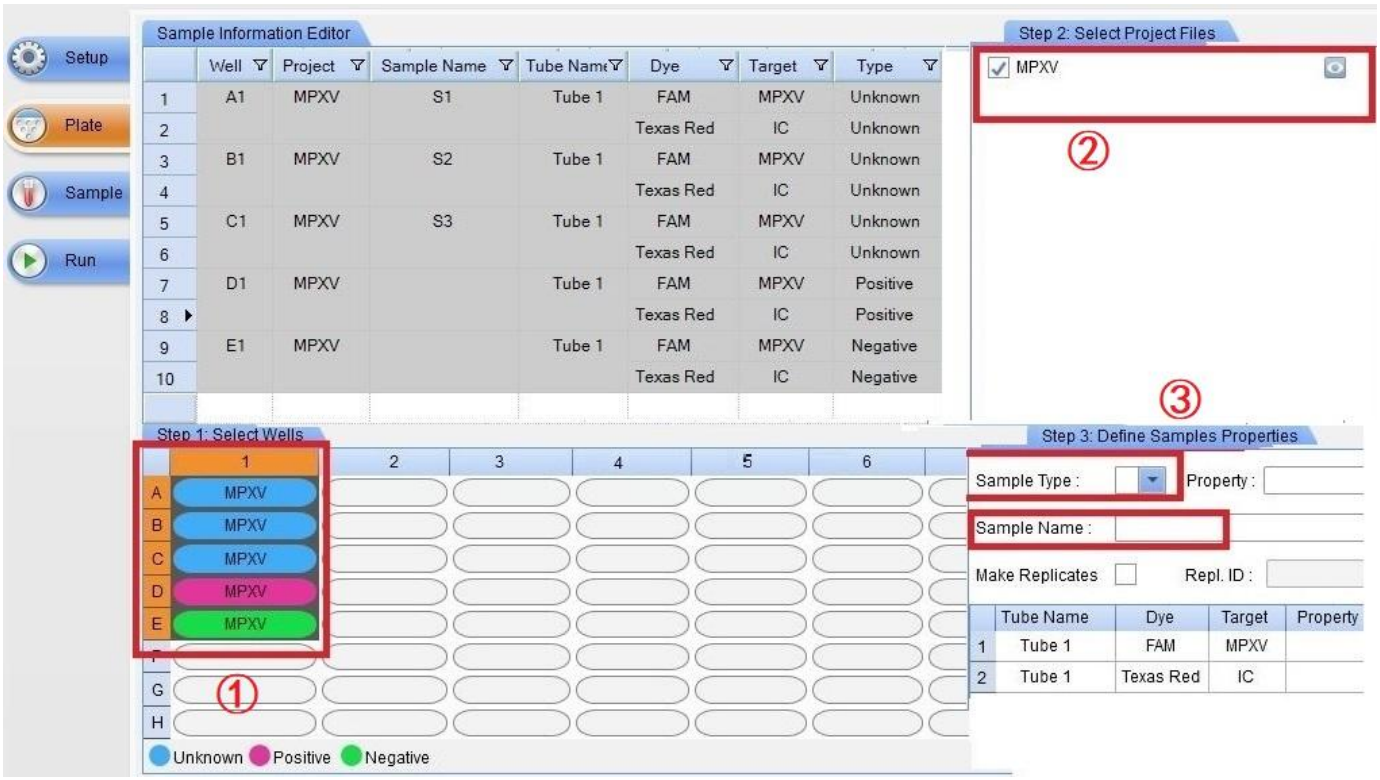
5. Click the button“Experiment”at the top to start a new experiment and then click “Experiment Wizard” to enter a new interface.



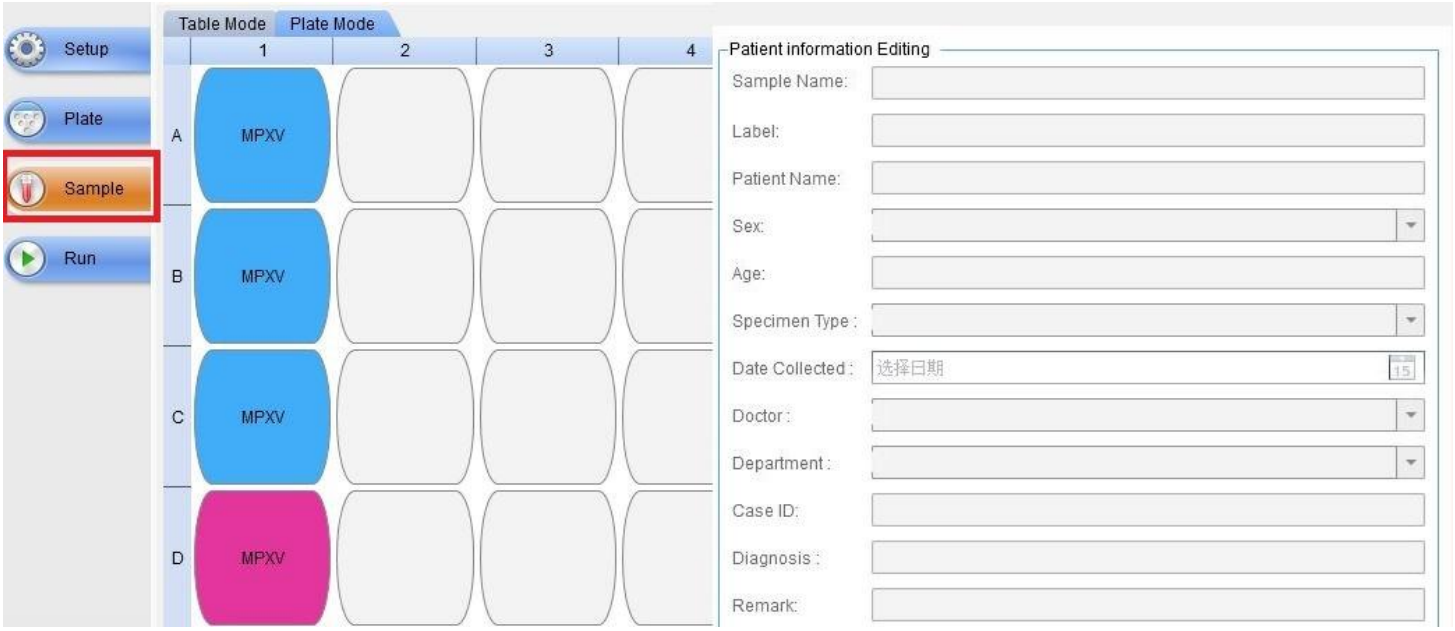
6. In the new interface, select “Setup”, and then enter the Name and choose the Save Path, Type, Temperature Control Mode and Hot-lid Mode as shown in the picture below.



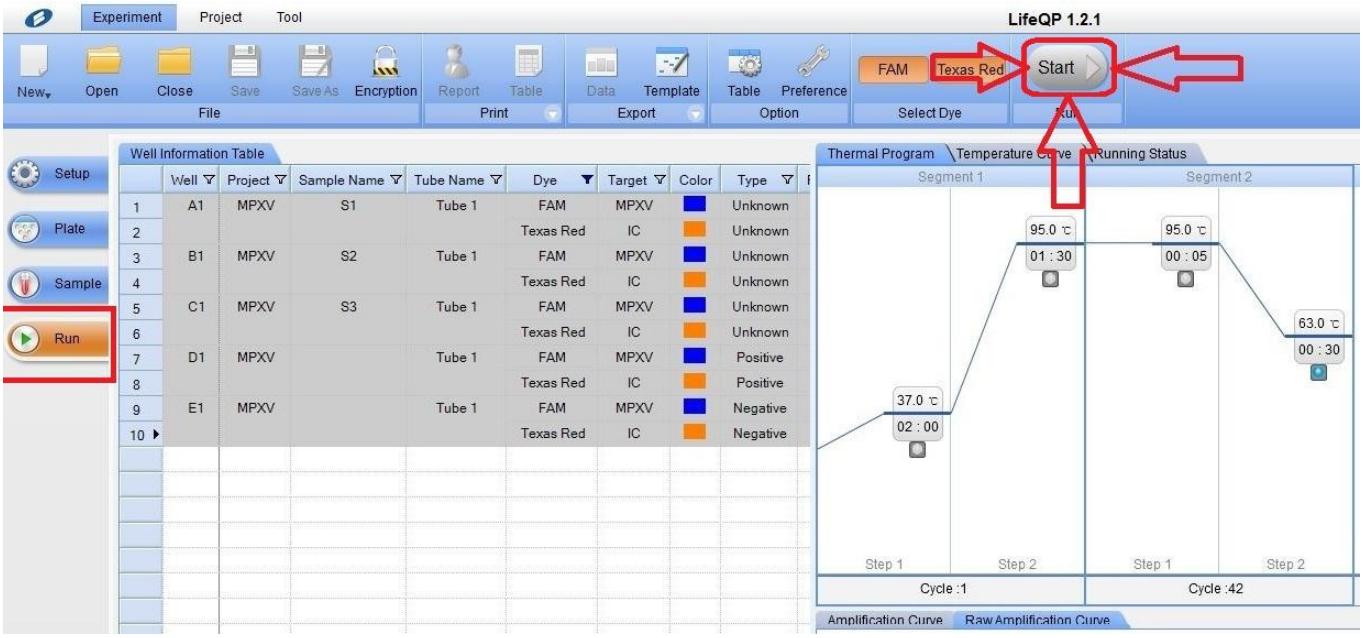
7. Choose “plate” on the right side to enter a new interface, select the well where the tubes are(①), and then choose the newly established project(MPXV) (②). After that, choose the sample type and enter the sample name. If in the tubes are samples, choose “Unknown”; if in the tubes are negative controls or positive controls, choose “Negative” or “Positive”. (③)



8. You can edit sample information in the “sample” column;



9. Click on “run” column to check if the relevant information is correct, and then click “Start” button in the upper right corner to start the experiment.


Experiment
Project
Tool
LifeQP 1.2.1
New
Open
Close
Save
Save As
Encryption
Report
Table
Data
Template
Table
Preference
FAM
Texas Red
Start
File
Print
Export
Option
Select Dye
Setup
Plate
Sample
Run
Well Information Table
Well
Project
Sample Name
Tube Name
Dye
Target
Color
Type
A1 MPXV S1 Tube 1 FAM MPXV Unknown
Texas Red IC Unknown
B1 MPXV S2 Tube 1 FAM MPXV Unknown
Texas Red IC Unknown
C1 MPXV S3 Tube 1 FAM MPXV Unknown
Texas Red IC Unknown
D1 MPXV Tube 1 FAM MPXV Positive
Texas Red IC Positive
E1 MPXV Tube 1 FAM MPXV Negative
Texas Red IC Negative
Thermal Program
Temperature Curve
Running Status
Segment 1
Segment 2
37.0 ℃
02 : 00
95.0 ℃
01 : 30
95.0 ℃
00 : 05
63.0 ℃
00 : 30
Step 1
Step 2
Step 1
Step 2
Cycle :1
Cycle :42
Amplification Curve
Raw Amplification Curve