CE

Monkeypox Virus Nucleic Acid Detection Kit (Real-Time PCR)

Revision:A/1

【Product Name】

Monkeypox Virus Nucleic Acid Detection Kit (Real-Time PCR)

【Cat. No.】

DV106

【Packaging Specifications】

48 tests/kit 200 tests/kit

【Intended Use】

This kit is suitable for the qualitative detection of monkeypox virus nucleic acids extracted from clinical samples such as nasal swabs, oropharyngeal swab, saliva, urine, skin lesion tissue, exudate, and blood, etc.

The test results of this kit are for clinical reference only and should not be used as the only standard for clinical diagnosis.It is suggested to make a comprehensive analysis combined with clinical symptoms and other laboratory testing methods.

The laboratory personnel for testing with the kit should be professionally trained with gene amplification or molecular biology detection and qualified for related experimental operations. Biosafety protective equipment and programs are required for the laboratories.

【Principles】

The kit is designed for detecting monkeypox virus nucleic acids in specimens using multiplex real-time PCR technology with primers and probes targeting the conserved regions of F3L gene of monkeypox virus. Simultaneously, this kit contains an endogenous control (The internal control *RNase-P* gene is detected by VIC channel) to monitor the process of specimen collection, nucleic acid extraction and PCR and reduce false negative results.

【Kit Contents】

Component Name	Main Constituents	Specifications and Quantity （48 tests）	Specifications and Quantity （200 tests）
MPXV PCR Reaction Mix	Taq DNA polymerase, uracil-DNA glycosylase，Primers and probes of F3L gene of monkeypox virus and the internal control-*RNase P* gene, *etc*.	960 μL×1 tube	1000 μL×4 tube
MPXV Positive Control	Plasmid with F3L gene of monkeypox virus and the internal control-RNase P	50 μL×1 tube	200 μL×1 tube

	gene		
MPXV Negative Control	Nucleic-free Water	50 μL×1 tube	200 μL×1 tube

Note: Components from different lots should not be mixed for use.

【Storage Conditions and Shelf Life】

Store the kit at -20±5°C away from light for 12 months.

Ship the kit at low temperature. Dry ice should be used for long-distance shipping; Avoid repeated freeze-thaw cycles (The number of freeze-thaw cycles should be fewer than 10).

Manufacture date and expiration date are shown on the label.

【Instrument】

Validated Instrument in-house: ABI 7500 and StepOne Plus Real-Time PCR instrument

Instruments used by customers with revised interpretation for test results : ABI QuantStudio 3, Bio-Rad CFX96.

Please contact our Technical Support team for other instruments.

【 Specimen Requirements】

1. Acceptable specimen types: nasal swabs, oropharyngeal swab, saliva, urine, skin lesion tissue, exudate, and blood, etc.

2. Sampling of specimen: Follow the local CDC guidelines and manufacturer's protocol for specimen sampling.

3. Specimen storage and shipping: National guidelines should be followed for specimen storage and shipping within national borders while International Transport Regulations should be complied with for international shipments.

If the specimen is stored in *TransGuard*™ Disposable Virus Sampling Tube (ES101), it can be stored at ambient temperature for one week.

【Test Method】

1. Specimen Preparation (Specimen Preparation Area)

Pipet 200 μL of specimen for nucleic acid extraction. Extracted nucleic acids can be used directly for detection. If the extracted nucleic acids is not for the subsequent detection after extraction immediately, it can be stored at -70ºC. Avoid repeated freeze-thaw cycles. If necessary, it is recommended to use EasyPure® Viral DNA/RNA Kit (ER201), MagicPure® Viral DNA/RNA Kit (EC301), or TS-32/96 Automated Nucleic Acid Extractor with MagicPure® 32/96 Viral DNA/RNA Kit manufactured by TransGen for DNA extraction.

2. Reagent Preparation: (Reagent Preparation Area)

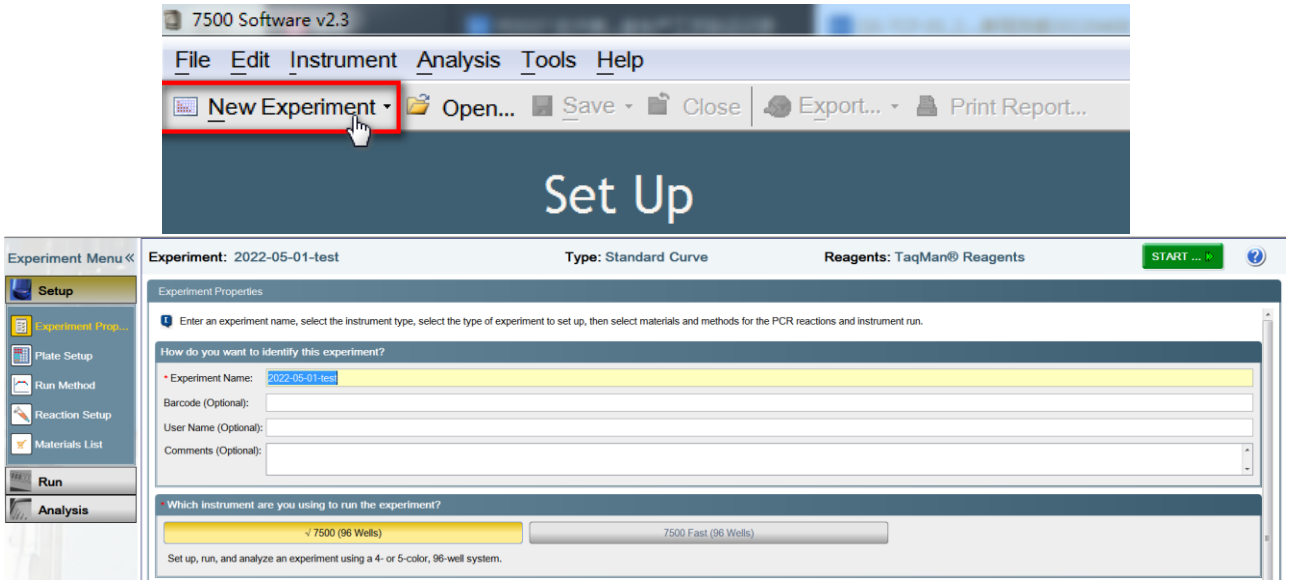
Thaw MPXV PCR Reaction Mix at room temperature. Mix thoroughly to ensure homogeneity, and then centrifuge briefly. It is recommended to set up a negative and positive control for each test. Dispense equal 20 μL into each microcentrifuge tube, and transfer to the Specimen Handling Area.

3. Specimen Addition (Specimen handling area)

Add 5 μL of extracted specimen nucleic acid, Positive Control, and Negative Control to the aliquoted system, to reach a total reaction volume of 25 μL. Tightly cap the reaction tube, then centrifuge briefly at low speed, and move to the Amplification and Analysis Area.

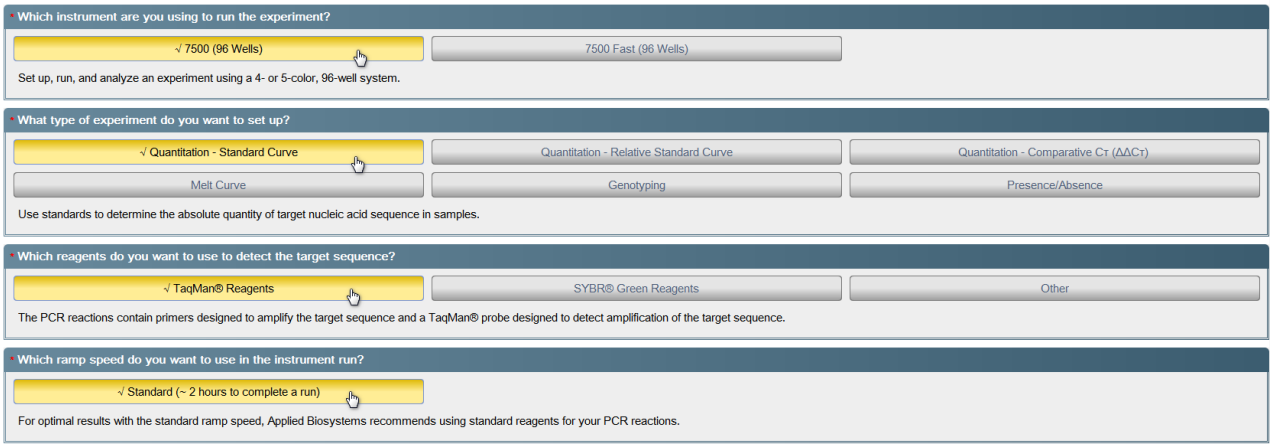
4. PCR Amplification (Amplification and Analysis Area)

Place the PCR tube in sequence into the PCR instrument. After starting 7500 Software v2.0.5, click New Experiment to open the Setup menu. In the Experiment Properties screen, enter an experiment name such as "2022-05-01-test" to identify your experiment.



Select **7500 (96 Wells)** for the instrument type, and select **Quantitation-Standard Curve** for the experiment type.

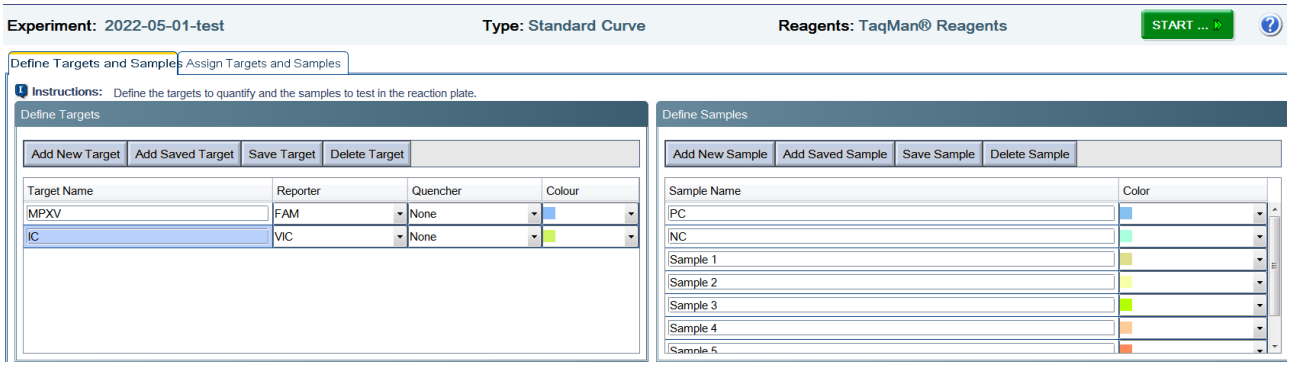
Select **TaqMan Reagents** for the reagents, and select **Standard (~2 hours to complete a run)** for the ramp speed.



Click “Plate Setup” icon. In the **Define Targets and Samples** screen, click the **Target Name** cell, then enter **MPXV**.

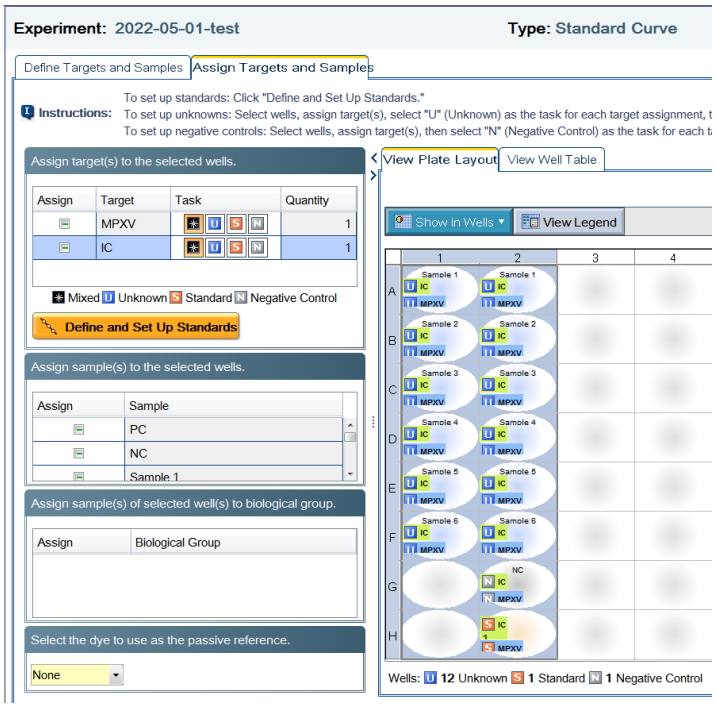
In the Reporter drop-down list, select **FAM**. In the Quencher drop-down list, select **None**. In the Color field, leave the default.

In the second row, click the **Target Name** cell, then enter IC. In the Reporter drop-down list, select **VIC**. In the Quencher drop-down list, select **None**. In the Color field, leave the default.



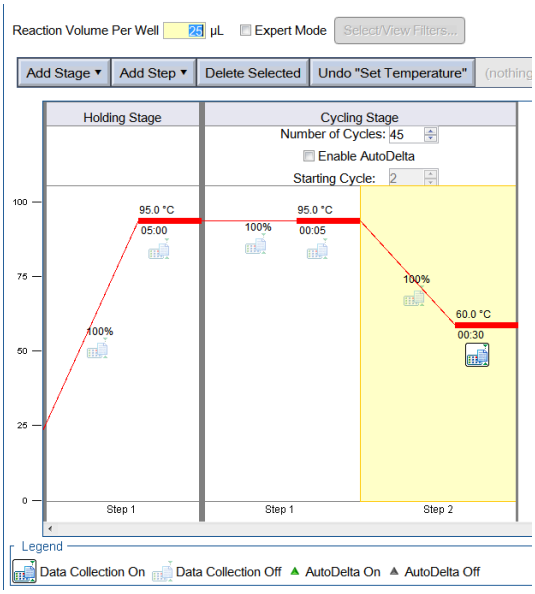
Click **Assign Targets and Sample** tab. Follow the Instructions in the window to set up standards, unknowns and negative controls. Select **None** for passive reference.

Click **Run Method**. In the **Run Method** screen, select either the **Graphical View** tab (default) or the **Tabular View**. Click the **Reaction Volume Per Well** field, then enter **25 μL**. Configure PCR protocol as shown in the table below. Review the thermal profile. After confirming that it is correct, click **Start the instrument run.**



STEPS	TEMPERATURE	REACTION TIME	CYCLES
Initial denaturation	95℃	5 min	1
Denaturation	95℃	5 sec	45
Annealing, extension and fluorescent signal collection*	60℃	30 sec*	

* Fluorescent signal should be collected during this step through the FAM and VIC channels.



5. Result Analysis

The results are automatically saved after the reaction. Then analyze the amplification curves of the target genes and internal control gene separately. According to the analysis of the image, adjust Baseline's Start value, End value and Threshold value. Click Analyze for analysis, and then record the qualitative results under the Plate window. (As for ABI 7500, the user can adjust manually according to the actual conditions to ensure that all the baselines for the curves are flat. For instance, the Start value can be set from 3 to 15, and End value can be set at 5 to 20. Threshold value should be set right above the summit of the amplification curve of negative control.)

6. Quality Control (Evaluation of Experiment Effectiveness)

Each control in the kit should meet the following requirements, otherwise the experiment is invalid.

	Positive Control	Negative Control
FAM channel（MPXV）	Typical sigmoidal curve，Ct ≤ 32	No Ct value or Ct>40
VIC channel（Internal Standard gene）	Typical sigmoidal curve，and Ct ≤ 32	No Ct value or Ct>40

【Reference Ct value for positive result】

The reference Ct value to determine target gene as positive is set at 38. The internal control for Ct value is 38.

【Interpretation for Test Results】

1. If a typical sigmoidal curve is observed in VIC channel of the specimen and Ct≦38, the results can be determined as the table below.

Ct value / Channel	Ct≤38	38<Ct≤40(in a sigmoidal shape)	Ct>40
FAM channel (MPXV)	Positive	Suspected positive	Negative

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For specimens tested as positive, when the Ct values of a target gene are between 38 and 40, it is necessary to observe if the amplification curve of the target gene is in sigmoidal shape. If not, the specimen should be regarded as suspected positive.

2. If the Ct value of VIC channel is higher than 38 without showing an apparent sigmoidal amplification curve, the causes can be listed as following:

1) PCR inhibitors exist in the specimen. It is suggested to dilute the specimen before test.
2) The operation of nucleic acid extraction is flawed. It is suggested to repeat nucleic acid extraction for the test.
3) Eligible specimens were not obtained in the processing procedures or specimens have been degraded during transportation and storage. It is suggested to perform sampling again.

【Limitations of Detection Method】

1. The test result is provided for reference only in clinical practice, but it cannot be the sole evidence for diagnosis.
2. Negative results can be caused by low quality of nucleic acids extracted from the specimens, improper storage conditions of extracted nucleic acids solution, inappropriate storage period, inhibitors in the specimen, nucleic acid degradation, *etc*.
3. False negative or false positive results are likely to be caused by inappropriate collecting, transportation and handling of specimens, or unsuitable experiment operation and environment. Other clinical observations and relative information should be combined for determination. Conduct the detection again when necessary.
4. False negative results may occur by sequence changes of target sequence of MPXV due to mutations or other reasons.

【Product Performance】

1. Minimum detection limit: 500 copies/mL.
2. Accuracy

The positive detection rate should be 100%. The negative detection rate for negative control should be 100%.

3. Analytical Specificity

No Cross with smallpox virus, vaccinia virus, Treponema pallidum, HIV virus, Epstein-Barr virus, Parvovirus B19, Herpes zoster virus, Influenza A virus (H1N1, H3N2, H5N1, H7N9), Influenza B virus (Yamagata, Victoria), Parainfluenza virus, Mycoplasma pneumoniae, Toxoplasma gondii, Rubella virus, Cytomegalovirus, Herpes simplex virus type II, seven Human coronavirus(SARS-CoV-2, HCoV-HKU1, HCoV-229E, HCoV-OC43, HCoV-NL63,MERS-COV and SARS-COV), Enterovirus virus(EV71,CA16) and Streptococcus pyogenes .

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The reference standards with and without mucin were both found positive. The negative samples were tested as negative.

4. Precision

Quantitative fluorescence PCR is used with negative samples, limited positive samples, and strong positive DNA samples. The results indicate that the negative detection rate of the negative samples is 100%; the positive detection rate of the strong positive samples and the limited positive samples are 100% and ≥95% respectively.

【Precautions】

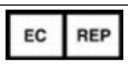
1. Please read the manual carefully before test and follow the protocol strictly.
2. Set both positive and negative controls for each test.
3. Test analysts should be trained by professionals and must perform operation in labs following safety guidelines and wear personal protective equipment.
4. The kits should avoid light for storage to protect the fluorophore from decay. All the centrifuge tubes, tips should be autoclaved to ensure DNase and RNase free.
5. Separate laboratory areas rigorously and perform the procedures in the predefined areas. To avoid cross contamination, all materials used in their designated area should not be moved or used in other areas. False positive results can be caused when cross contamination is not controlled during the sample treatment process.
6. All lab workbench and supplies, such as pipettes, centrifuges, PCR cyclers should be disinfected using 1% hypochlorous acid or UV light for 25-30 minutes.
7. After amplification, take out the reaction tubes and seal in a specially designed plastic bag to dispose in a designated area.
8. The test specimens involved in this kit should be considered as infectious substances, and their treatment and handling must meet the relevant regulations of the General Guidelines for Biosafety of Microbiology and Biomedical Laboratories and the Medical Waste Management Regulations issued by of the Ministry of Health.

【References】

1. World Health Organization. Surveillance, case investigation and contact tracing for Monkeypox.2022.
2. World Health Organization. Managing epidemics: Key facts aboiut major deadly diseases. 2018.
3. World Health Organization. WHO Guidance on regulations for the transport of infectious substances 2021-2022.
4. World Health Organization. Laboratory Biosafety Manual, 4th edition. 2020.
5. McCollum AM, Damon IK. Human Monkeypox. Clinical Infectious Diseases. 2014 Jan 15;58(2):260–7.

6. World Health Organization. International Health Regulations (2005) Third Edition.
7. Li Y, Zhao H, Wilkins K, Hughes C, Damon IK. Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. Journal of Virological Methods. 2010 Oct;169(1):223–7.
8. Schroeder K, Nitsche A. Multicolour, multiplex real-time PCR assay for the detection of human-pathogenic poxviruses. Molecular and Cellular Probes. 2010 Apr;24(2):110–3.

【Symbols and Interpretations】

				
For in vitro diagnostic use only	Attention, see instruction for use	Do not use if package is damaged	Limiting temperature	Do not reuse
				
Afraid of the sun	Manufacturer	Date of production	Validity	Batch code
				
Conformity of European	Authorized Representative	Tests per kit	Keep dry	Catalog

【Basic information】

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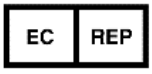

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