Human Monkeypox virus nucleic acid Test Kit (RT-PCR)

Catalog No: E-HD-P001
50/100/150T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Phone: 240-252-7368(USA) 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please refer to specific expiry date from label on the side of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

The Monkeypox virus nucleic acid test kit is performed on the applicable instrument to detect DNA extracted from lesion exudate specimens. It utilizes the monkeypox virus conserved sequence of F3L gene as multiplex real time PCR amplification target regions.

The fluorescent quantitative PCR instrument can automatically draw a real-time amplification curve based on the detected fluorescent signal, so as to realize the qualitative detection of the monkeypox virus at the nucleic acid level. The multiplex real time PCR detection system includes a pair of primers and probes for internal control (HBB). The positive control and negative control included in the kit can be used as an external control in every test run. The results of internal and external control can be used to monitor correct specimen collection, handling and real time PCR process.

Intended use

The Monkeypox virus nucleic acid Test Kit is a real-time polymerase chain reaction (PCR) test intended for the qualitative detection of monkeypox virus in lesion exudate and serum specimens.

Kit components

The kit should be stored at -30 to -15℃ protected from light until the expiration date printed on the pouch. Repeated freezing and thawing (≥10 cycles) should be avoided.

Item	Specifications	Main Ingredients	
PCR reaction buffer	1/2/3 mL	Buffer, dNTPs, Primers, Probes, Taq DNA polymerase.	E-HD-P001
Positive Control	0.1/0.2/0.3 mL	Synthetic plasmid containing target genes.	E-HD-P001
Negative Control	0.1/0.2/0.3 mL	DEPC-Treated ddH2O.	E-HD-P001
Manual	1 copy	/	E-HD-P001
Extraction Buffer*	1/2/3mL	EDTA, Chelate resins	optional

*The components of this reagent are optional, not the contents of the kit.

Note: Do not mix the components from different batches of kits.

Applicable Instrument

Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument

Materials needed but not provided

- Biosafety cabinet
- Personal protective equipment (PPE)
- General laboratory equipment (e.g.tube racks)
- Real time PCR system
- Nucleic acid extraction kit and instrument
- Pulse centrifuge
- Vortex mixer
- Real time PCR reaction tubes (0.2 mL)
- Ice-container
- Transfer pipettes (0.5 μL-1000 μL)
- Sterile tips for transfer pipettes
- Sterile tubes
- Biohazard waste container
- Refrigerator and freezer

Sample collection and preparation

1. Collecting Specimen

Wipe the lesion with a swab, rotate and stay for 10-30 seconds to allow the swab to fully absorb secretions. Specimen should be collected into sterile, labeled tubes and shipped per testing laboratory requirements.

2. Specimen Storage

The collected specimen should be tested as soon as possible. If it is not tested immediately, the samples to be tested should be stored at 2-8 ℃ for no more than 24 hours; at -20 ℃ for no more than three months, below -70 ℃, they can be stored for a long time.

Note: Inappropriate storage of specimens as well as frequent thawing and re- freezing can damage the specimens and should be avoided; otherwise false negative results may occur.

Assay procedure

Note: All work areas should be in separate (Reagent storage and preparation area, Specimen preparation area and Amplification area).

All necessary safety precautions should be taken according to the laboratory guidelines. Precautions must also be taken to prevent cross-contamination of specimens.

1. Reagent Storage and Preparation Area

1.1 Thaw the kit components at 4℃ to 30℃.

1.2 Thaw the PCR reaction buffer at room temperature, fully thaw, shake and mix well, centrifuge at low speed for a few seconds, and then divide the solution into PCR reaction tubes (20μL/tube) according to the number of samples to be amplified (sufficient for the number of patient samples and controls). Cap the PCR reaction tubes with care, ensure that the caps are placed correctly, and transfer to the specimen preparation area, store in a refrigerator at 4 ℃ away from light.

Note: It is important to thoroughly vortex the reagents. It is recommended that always taking account a surplus of 10% to compensate for pipetting inaccuracies.

2. Specimens Preparation Area

2.1 DNA extraction

DNA is extracted according to the instructions for use of the extraction kit.

2.2 Add 1 mL of normal saline to the sample collection tube, shake the cotton swab sufficiently to wash it, then squeeze the cotton swab against the tube wall and discard it, transfer 500 μL of liquid to a 1.5 mL tube, centrifuge at 12,000 rpm for 5 minutes, discard the supernatant, collect the sediment.

2.3 Add 1 mL of normal saline to the sediment, shake on a vortex shaker to disperse the sediment (use a pipette tip to gently disperse the sediment if necessary), centrifuge at 12,000 rpm for 5 minutes, discard the supernatant, and collect the sediment.

2.4 Add 50 μL of the prepared extraction buffer to the sediment, shake on a vortex shaker to disperse the sediment (use a pipette tip to gently disperse the sediment if necessary), dry or water bath at 100 ℃ for 10 minutes, centrifuge at 12,000 rpm for 5 minutes, and remove the supernatant solution for PCR reactions (Be careful not to touch the sediment at the bottom of the tube when pipetting).

2.5 The extracted DNA should be used for detection in time, otherwise it should be stored at -20 ℃. When using again, the extracted DNA should be fully thawed and centrifuged at 12,000 rpm for 5 minutes, and the supernatant should be used for PCR reaction.

2.6 If the extracted DNA is not used immediately, it can be stored at 2-8 ℃ for no more than 24 hours.

3. Addition of samples

3.1 Pipette 5 μL of each purified DNA sample, negative control and positive control into the corresponding PCR reaction tube containing the PCR reaction buffer.
Note: Change the pipette tip with every step.

3.2 Cap the PCR reaction tubes with care; ensure that the caps are placed correctly.

3.3 Vortex the PCR reaction tubes briefly and centrifuge to collect the solutions at the bottom.

4. Amplification Area

4.1 Put the reaction tubes on a PCR instrument, set up and run the following cycling protocol.

Step		Cycles	T (℃)	Time(s)
Step 1	Initial denaturation	1	95℃	300
Step 2	Denaturation	40	95℃	10
Step 3	Annealing, extension and testing		60℃	30

Note: Reaction volume was set at 25μL.

4.2 Settings of detection fluorescence

Channel	Target Gene
FAM	F3L gene of Monkeypox virus
JOE	HBB

a) Please set the internal reference parameter of fluorescence of the instrument to "None". For example: for ABI 7500, please set "Passive Reference" to "None".

b) Start the PCR cycler according to its user manual.

Result analysis

ABI 7500: Set Baseline to 3-15 (Baseline Cycler can be changed within a certain range according to the actual situation), and the fluorescence threshold (Threshold) setting principle is that the threshold line just exceeds the maximum value of the negative control amplification curve (irregular noise line) point, and the Ct value is displayed as Undet. Use the instrument software to automatically analyze the results.

Quality control

1. Negative control: no obvious S-shaped amplification curve, and Ct value is shown as Undet.
2. Positive control: both channels have typical S-shaped positive amplification curves, and the Ct value is less than or equal to 30.

Note: The above two conditions must be satisfied at the same time, otherwise, this test is invalid.

Reference value

1. Ct value is displayed as undet, negative result.
2. Ct value < 38, positive result.

Interpretation of test results

On the premise that the experiment is valid, the test results are judged according to Table below.

Table.1 Ct value of each fluorescence channel and judgment of negative and positive results

Fluorescence channel (target gene)	**Ct value**	**Interpretation**
JOE (internal reference)	Ct =40（or undet）	Negative
	Ct < 40，With obvious S-type amplification curve	Positive
FAM（Monkeypox virus）	Ct =40（or undet）	Negative
	Ct < 38，With obvious S-type amplification curve	Positive
	38≤ Ct < 40，With obvious S-type amplification curve	To detect the gray area, the measurement should be repeated once. If the Ct value of the retest result is less than 40, it is judged to be positive; the Ct value of the retest result is 40 (or displayed as undet), and it is judged to be a negative result;

Table.2 Determination of Monkeypox virus test results

Internal reference	**Monkeypox virus**	**Interpretation**
+	-	Negative
+ or -	+	Positive
-	-	PCR failure, there may be inhibitors in the DNA sample preparation process. It is recommended to dilute the DNA by 10 or 100 times and repeat the measurement. If there is a fluorescent signal, follow the above judgment result, otherwise, re-sample.

Note: “+” positive, “-” negative.

Performance characteristics

1. Limit of Detection (LOD)

Components	LOD
Monkeypox virus	200 copies/μL

2. Inclusivity

The inclusivity of the Monkeypox virus nucleic acid Test Kit was evaluated using 51 monkeypox viruses representing temporal, geographic, and genetic diversity. Parts of inclusivity evaluation results are presented in the following tables.

Note: Please contact the manufacturer to obtain the complete table of data.

Table.3 Inclusivity Evaluation Results of Monkeypox Virus

Accession	Isolate/Strain	Accession	Isolate/Strain
AY603973.1	Monkeypox isolate MPXV-WRAIR7-61	KJ642615.1	Monkeypox isolate W- Nigeria
DQ011153.1	Monkeypox isolate USA_2003_044	MG693723.1	Monkeypox isolate MPXV_Nig_2017_297957, partialgenome
HM172544.1	Monkeypox isolate Zaire1979-005	MN648051.1	Monkeypox isolate Israel_2018
HQ857562.1	Monkeypox isolate V79-I-005	JX878407.1	Monkeypox isolate DRC06- 0950
KP849470.1	Monkeypox isolate Coted'Ivoire_1971	KP849469.1	Monkeypox isolate Boende_DRC_2008
JX878409.1	Monkeypox isolate DRC06-0999	KJ642617.1	Monkeypox isolate Nigeria-SE-1971
KC257459.1	Monkeypox isolate Sudan2005_01	MG693725.1	Monkeypox isolate MPX_Nig_2017_298481, partialgenome
KJ642612.1	Monkeypox isolate Ikubi		

3. Analytical Specificity

3.1 **Cross-reactivity:** For Monkeypox virus nucleic acid Test Kit, there are also no cross-reaction with organisms shown as follows.

Table.4 Cross-reactivity

Components	LOD
Monkeypox virus	Positive
Varicella-Zoster virus	Negative
Bovine papular stomatitis virus	Negative
Cowpox virus	Negative
Vaccinia virus	Negative

Yaba monkey tumor virus	Negative
Variola virus	Negative
Tanapox virus	Negative
Pseudocowpox virus	Negative
Rabbitpox virus	Negative
Orf-Virus	Negative
Human molluscum contagiosum virus	Negative

3.2 **Interfering substances:** The final concentration of interfering substances in the following table have no significant interference with the detection results of the kit.

Table.5 Possible interfering substances in specimens

Interfering substances	**Final concentration**	**Interfering substances**	**Final concentration**
hydroxymezoline hydrochloride	100 μg/mL	budesonide	320 μg/mL
dexamethasone	50 μg/mL	beniferin	125 μg/mL
cefmenoxime hydrochloride	50 μg/mL	tobramycin	100 μg/mL
oseltamivir,	100 μg/mL	beclometrasone	50 μg/mL
zanamivir	100 μg/mL	flunicasone	100 μg/mL
ribavirin	100 μg/mL	momethasone	100 μg/mL
azithromycin	100 μg/mL	fluticasone	200 μg/mL
α-interferon	300 U/mL	histamine dihydrochloride	200 μg/mL
peramivir	100 μg/mL	lopenavir	100 μg/mL
mupiroxacin	100 μg/ml	triamcinolone	100 μg/mL
litonavir	100 μg/mL	abidor	100 μg/mL
urea	100 μg/mL	sodium chloride	60 μg/ml
purified mucin	20 μg/mL	heme	10 μg/mL
anhydrous ethanol	20% (v/v)	human whole blood	20% (v/v)

Limitations

1. Performance has been evaluated with lesion exudate specimens only, using the instructions for use in the test kit.
2. Negative results do not preclude monkeypox virus infection
3. As with any molecular test, mutations within the target regions of this test kit could affect primer and/or probe binding resulting in a failure to detect the presence of a virus.
4. False negative results may occur if inadequate numbers of organisms are present in the specimen.
5. The test result dose not rule out the presence of other co-infection pathogens.

Note

1. This product is for scientific research use only.
2. Please read the instructions for use carefully before use, and strictly follow the instructions.
3. Do not interchange components in different batches of kits.
4. Do not use expired products or products with a broken aluminum foil.
5. The specimens must be treated as potential sources of infection, and must be performed using proper PPE against biological risk according to published guidelines and local regulations.
6. Avoid the liquid in contact with eyes and skin. If it splashes onto the skin or eyes, please wash immediately with plenty of water.
7. Cap the reagents immediately after use.
8. All kit components shall be completely thawed before use.
9. The master mix must be well mixed and placed in the ice-container.
10. Use separate pipette tip for each specimen to avoid cross-contamination of specimens which could cause erroneous results.
11. Please pay attention to the note of the biosafety cabinet during the operation procedures.
12. The test accuracy is affected by the specimen collection, storage and transport process.
13. Operate in the biosafety cabinet with clean disinfection and ultraviolet sterilization to prevent the outflow of aerosol and avoid harmful substances entering the respiratory tract.
14. Perform the test in partitions (Reagent storage and preparation area, Specimen preparation area and Amplification area) and prohibit cross-movement of personnel or equipment between areas.
15. Follow the standard biosafety guidelines for handling and disposal of potentially infective material.
16. If you have any questions or suggestions during use, please do not hesitate to contact the manufacturer.
17. Each reagent is optimized for use in the E-HD-P001. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-HD-P001 with different lot numbers.

Storage and expiry date

Storage: Store at -30 to -15℃. Must be frozen.

Expiry date: expiration date is on the packing box.