In vitro diagnostic medical devices

Asan qPCR Test® MPOX

1. LOT number and Expiry date
Refer to external labelling

2. Intended use
Asan qPCR Test® MPOX is an in vitro diagnostic medical device that helps diagnose the infection of MPOX and OPXV by qualifying MPOX genes (G2R genes) and Orthopox Virus (OPXV) genes (E9L genes) by real-time polymerase chain reaction (Real-time PCR) in skin lesions, skin swap samples, and blood of people suspected of being infected with Monkeypox Virus (MPOX).

3. How to use
(1) inspection principle
This product is a One-step PCR product using a real-time polymerase chain reaction (Real-time PCR), and uses the material required for the One-step PCR reaction in one tube. Using this, PCR for target genes is carried out from DNA extracted from the sample in the same tube.
The real-time PCR method uses primer & probe, in which the amplification product amplified by the primer binds to the probe, the polymerase decomposes the probe, and the fluorescence signal of the generated reporter eye is measured in real time. In this process, the fluorescence signal increases according to the amount of the amplification product. The DNA extraction process and whether the PCR reaction inhibitor in the extracted DNA is mixed can be confirmed by the Human ABL1 gene, an internal control (IC), and whether the sample is properly collected and extracted.
This product can qualitatively detect Monkeypox Virus and Orthopox Virus by simultaneously amplifying the gene G2R gene targeting the Empox virus and the gene E9L gene targeting the Orthopox virus and measuring fluorescence signals.

(2) Kit Components

	Components	Volume	Quantity
1	Onestep qPCR Master Mix	1.0 mL	1 tube
2	Primer/Probe Mix	500 µL	1 tube
3	Positive Control	250 µL	1 tube
4	Negative Control	250 µL	1 tube

※ Material and devices required but not provided
CFX 96™ Real-Time PCR Dectection system(Bio-Rad)
- Nucleic acid extraction system or kit
- Benchtop centrifuge with a rotor for 1.5/2.0 mL reaction tubes
- Centrifuge with a rotor for microtiter plates
- Vortex mixer
- Appropriate reaction plate or tube
- Pipettes
- Pipette tips with filters
- Disposible latex gloves

(3) Specimen preparation and storage
1) The available specimens are human skin lesions and skin swab specimens.
2) Samples stored in the transport medium should be recognized for up to 72 hours when refrigerated (2~8℃), and frozen under -70℃ for long-term storage.
3) Specimens can be stored for a long period of time, or test sensitivity can be reduced when freezing and thawing are repeated.
※ All clinical specimens are treated as likely to carry infectious pathogens.

4. Procedure
(1) Nucleic acid extraction
It is recommended to use the QIAamp DSP DNA Viral Mini Kit as a nucleic acid extraction reagent, and follow the manufacturer's instructions.

(2) How to test
[Preparation of reagent]
1) Open the box, and thaw each components
2) Lightly voltex each completely melted component and spin-down.

[Mix the components]
1) After considering the number of samples N (number of specimens + Positive Control 1EA + Negative Control 1EA + Extra 1EA) to be tested, prepare 15 ㎕ x N of reaction solution using Onestep qPCR Master Mix and Primer/Probe Mix as shown in the table below.

	components	1 Test Volume	N Test Volume
1	Onestep qPCR Master Mix	10 µL	10 µL × N
2	Primer/Probe Mix	5 µL	5 µL × N
3	Viral DNA Sample	5 µL	5 µL × N
	Total	20 µL	20 µL × N

2) Voltex the prepared reaction solution lightly, being careful not to create bubbles, and then spin down.
3) Dispense 15 µl of the prepared reaction solution into the PCR tube.
4) Dispense 5 µl of the extracted Viral DNA Sample, Positive Control (PC), and Negative Control (NC) into each PCR Tube. (To minimize contamination, work in the following order: NC > Sample > PC.

[Amplication of Real-time PCR]
1) After dispensing, spin down the PCR tube and place it into the PCR well.
2) Operate the equipment following the user manual instructions.
3) Refer to the Real-time PCR equipment settings in the following table.

<Setting of CFX96™ Dx System fluorescent>

Target	MPOX (G2R gene)	OPXV (E9L gene)	IC
Fluorophore	FAM	HEX	Cy5

<Setting of Real-time PCR condition>

	Steps	Temperature	Time	Cycle
1	Denaturation	95℃	20s	1
2	Pre-Denaturation	95℃	3s	5
3	Pre-Annealing/ Extension	60℃	30s	
4	Denaturation	95℃	3s	40
5	Annealing / Extension	60℃	20s	

5. Result analysis

When the inspection is completed, the appropriate experimental data of the analysis program is opened, the result analysis is performed, the Cut-off (Ct Value) value for the target gene is checked, and the threshold is set to 300 for the IC.

Target	Ct Value	Result
MPOX	Ct ≤ 36	Monkeypox virus positive
OPXV	Ct ≤ 36	Orthopox virus positive
IC	Ct ≤ 34	Internal control positive

<Overall results interpretation>

Case	MPOX	OPXV	IC	Interpretation
1	+	+	+/-	MPOX positive
2	-	+	+/-	OPXV positive, MPOX negative
3	-	-	+	Negative
4	-	-	-	Invalid/Re-test

- Even if the internal control material (IC) comes out negative, it may be determined as positive. When each detection gene is strong positive, the IC result value may be inhibited due to the high concentration of the detection gene, so even if the IC is negative, it is determined as positive.
- Invalid/Re-test: If the test is invalid, a retest is performed.

6. Quality control and validity of results

A minimum of one Negative Control and one Positive Control must be included in each run. All control wells must pass for the Real-time qPCR plate to be considered valid.

Target	MPOX (FAM)	OPXV (HEX)	IC (Cy5)
PC	Ct≤36	Ct≤36	Ct≤34
NC	N/A	N/A	N/A

7. Performance

(1) Analytical sensitivity – Limit of detection (LoD)

Monkeypox Virus (Mpox Virus Positivie Control, ZeptoMetrix No.NATMPXVPOS-6C) and Orthopox Virus (recombinant plasmid DNA) standards were diluted step by step and tested in five repetitions in one test with two lots, twice a day, for a total of 60 repetitions per concentration for three days to confirm the lowest concentration detected more than 95%.

Detection gene	Limit of detection (LoD)
	Detection concentration(Copies/μL)
Monkeypox virus	1.01 Copies/μL
Orthopox virus	10.32 Copies/μL

(2) Precision - Repeatability

Three samples of high concentration (100xLoD), medium concentration (10xLoD), and low concentration (2xLoD) were tested as a set by the same experimenter, twice daily, three times per test, over five days using one lot. The test confirmed that the Ct values detected within each test, between tests, and across test dates had a coefficient of variation (CV) of less than 5%, indicating effective repeatability.

(3) Precision – Reproducibility

Three samples of high concentration (100xLoD), medium concentration (10xLoD), and low concentration (2xLoD) were used as a set to assess reproducibility across locations, testers, and lots. Two testers across two laboratories tested two different lots twice daily for five days, with each test repeated three times. The results consistently showed 100% agreement between negative and positive outcomes, and the coefficient of variation (CV) for detected Ct values was less than 5%. This confirmed effective reproducibility.

(4) Competitive interference

In order to confirm the competitive interference between the analytes of Influenza A Virus, Influenza B Virus, Respiratory Synthetic Virus, and Adenovirus, the test was repeated 20 times with one lot product for each concentration under the following conditions.

As a result of the test, it was confirmed that it was suitable within the determination criteria, and it was confirmed that another target at a high concentration (100xLoD) did not affect the analysis sensitivity of the corresponding target at each low concentration (2xLoD).

Primer/Probe Mix		
Reference material	Combination	
Monkeypox Virus	Low Concentration(2xLoD)	High Concentration
Orthopox Virus	High Concentration	Low Concentration(2xLoD)

(5) Analytical specificity - cross-reaction

As a result of evaluating the presence or absence of cross-reaction by repeatedly testing 33 types of various microorganisms and viruses present in the sample three times in one lot of products and evaluating the presence or absence of cross-reaction at each sample, it was confirmed that no cross-reaction was performed at the corresponding analysis concentration for the remaining substances except for target genes detected in the product. The concentration was set as meaningful concentration in the experiment by referring to related papers and guidelines, and the experiment was conducted by selecting more than 10^5PFU/mL for virus and 10^6CFU/mL for bacteria.

No	Pathogen name	Concentration
1	Monkeypox Virus	Zeptometrix
2	Candida albicans	KCCM 11282
3	Neisseria gonorrhoeae	ATCC 43069
4	Neisseria sicca	KCCM 11892
5	Staphylococcus aureus	KCCM 12214
6	Staphylococcus epidermidis	KCCM 35494
7	Streptococcus agalactiae	KCCM 41599
8	Streptococcus equi	KCCM 12122
9	Streptococcus pyogenes	KCCM 11873
10	Yersinia enterocolitica	ATCC 27729
11	Rubivirus	ATCC VR-553
12	Dengue virus type 1	ATCC VR-1856
13	Dengue Plasma	Zeptometrix
14	Parvorius B 19	NIBSC 12/208
15	hEnterovirus	KBPV-18
16	hEnterovirus	KBPV-19
17	Dengue virus type 2	KBPV-VR-29
18	Dengue virus type 4	KBPV-VR-31

19	hEnterovirus	KBPV-23
20	hEnterovirus	KBPV-26
21	HSV-1	KBPV-57
22	HSV-2	KBPV-58
23	Chikungunya virus	NCCP 43132
25	MERS-Coronavirus (Florida/USA-2_Saudi Arabia_2014)	Zeptometrix
26	Human coronavirus HKU1	ATCC VR-3262SD
27	Human SARS Coronavirus	Zeptometrix
28	Adenovirus 1	ATCC VR-1
29	Adenovirus 4	ATCC VR-1572
30	Adenovirus 7	ATCC VR-7
31	Adenovirus 40	ATCC VR-931
32	Bacillus licheniformis	NCCP 16737
33	Bordetella pertussis	NCCP 12750

(6) Analytical specificity – interference response

Nine types of endogenous or exogenous substances were added to the samples and tested three times per sample. It was confirmed that there was no interference for 9 types of interfering substances up to the corresponding concentration.

No	Interference material	Concentration	Interference or not
1	Saline Nasal spray	10%(V/V)	N.A
2	Zanamivir	3mg/ml	N.A
3	Bilirubin, Conjugated	0.05mg/ml	N.A
4	Albumin	0.24g/ml	N.A
5	Haemoglobin	2mg/ml	N.A
6	Biotin	300μg/ml	N.A
7	Tobramycine	24μg/ml	N.A
8	Mucin	0.1mg/ml	N.A
9	Human genomic DNA	10μg/ml	N.A

N.A: Not available

8. Storage method and period of use

(1) Store the kit in its original sealed packaging at -20℃. or below, away from direct sunlight. Kit contents are stable until the expiration date printed on the outer box.

(2) The number of freeze/thaw cycles is limited to four

9. Precautions

[General precautions]

(1) This product is for in vitro diagnostic use only.

(2) This test should only be conducted by trained professionals.

(3) Before starting the assay, thoroughly read the user manual and follow the instructions for use.

(4) All specimens collected from humans should be considered potentially infectious. Follow Good Laboratory Practice (GLP) when handling samples to prevent contamination.

(5) Avoid contact of this product with skin, eyes, or mucous membranes. If contact occurs, rinse immediately with plenty of water.

(6) Improper control of sample cross-contamination during handling and processing may lead to false positive results.

[Precautions for storage]

(1) Store the product below -20℃.

(2) Do not use products beyond their expiration date.

[Precautions when test]

(1) Before use, visually inspect each component of the product for leaks. Do not use if a leak is detected.

(2) Do not mix this product with products from other lots during use.

(3) Avoid repeated freezing and thawing of the product, as it may affect test results.

(4) This product is optimized for use with the recommended real-time gene amplification device. Use with other equipment may result in incorrect results.

(5) Take care to prevent contamination of product components by nucleic acid extracts, PCR products, or positive controls. We recommend using filter tips to prevent contamination.

(6) In case of spills onto equipment, clean according to the equipment manual and remove contamination from surfaces.

(7) All samples used in testing are potentially infectious. Use appropriate protective equipment and conduct testing in facilities such as biosafety cabinets of Biosafety Level 2 or higher.

(8) Improper collection, transport, or handling of samples may affect test results. Handle samples with caution.

[Precautions for determining results]

(1) Do not utilize test results obtained with this product for disease diagnosis. Diagnosis should be based on test results, clinical findings, and other clinical data evaluated by a healthcare professional.

(2) A negative result alone may not rule out MPOX and OPXV infection if the sample's viral concentration is below the detection limit or if there are amplification inhibitors present.

(3) Sensitivity may decrease if there are mutations in the primer and probe binding regions included in the product.

(4) This test does not exclude diseases caused by other bacterial or viral pathogens.

[Precautions for disposal]

(1) Dispose of products and samples following guidelines and methods outlined in the Waste Management Act.

10. Creation and revision date

Date of preparation : 2024.12

[Product name] High-risk infectious agent genetic testing reagent
[Classification number (grade)] K05030.01(3)
[Model name] Asan qPCR Test® MPOX

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ISO13485:2016
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