RADI Monkeypox Detection KIT

REF RV015

1. Intended use

The RADI Monkeypox Detection KIT is an *in vitro* diagnostic medical device, based on Real-time PCR technology to detect the Monkeypox virus DNA.
The kit is intended for the presumptive qualitative detection of nucleic acid extracted from the Monkeypox virus in human skin lesion, crust or swab specimens obtained from patients with signs and symptoms of Monkeypox.
The assay is for use by a laboratory professional trained to use real-time PCR in a laboratory.

2. Precautions and Warning

1) For in vitro diagnostic use (IVD) only.
2) This assay needs to be carried out by trained personnel.
3) Please wear disposable gloves when handling.
4) Prior to commencing an IVD test, make sure all the reagents are melted well on the operating room temperature.
5) When the control value is out of the expected range (see "**10. Quality Control**"), it is indicative of instability or deterioration of the kit.
6) To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area.
7) To avoid contamination from the positive control (PC), pipette the PC last.
8) Do not use the kit after its expiration date.
9) Always use sterile pipette tips with filters and use a new tip every time a volume is dispensed.
10) Avoid eyes, skin and clothing contact with reagents. In case of any contact, flush with flowing water.
11) Follow standard precautions for infectious waste management. All patient specimens and positive controls should be considered to be potentially infectious and handled with precautions.
12) Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
13) Handle all specimens considering as infectious and follow the safe laboratory procedures[3].
14) Specimen processing should be performed in accordance with national biological safety regulations.
15) DNA should be maintained on cold block or on ice during preparation to ensure stability.
16) Dispose of used kit reagents and human specimens according to local, state, and federal regulations.
17) Do not reuse disposable materials after use.
18) Do not pool reagents from different lots or from different kits of the same lot.

3. Kit Components

Materials Provided (100 tests/kit)

Lid Color of Tube	Component	Volume(㎕)
Brown	Monkeypox Primer & Probe mixture	500
Yellow	2X RADI FAST MasterMix	1,000
Red	Monkeypox Positive control	300
Blue	RNase free water	1,000

4. Instruments and materials required, but not provided

- Micropipette
- Sterilized pipette tips with filter barriers
- Centrifuge
- Disposable powder-free gloves
- Real Time PCR machine
  - ✓ CFX96 Real-Time PCR Detection System, Bio-rad (Cat.no: 1845097 / S/W ver.: 3.1)
- Consumables relating the RT-PCR
  - ✓ 96-Well PCR Plate
  - ✓ 1.5 mL microcentrifuge tubes (DNase/RNase free)
  - ✓ PCR Plate Sealing film (adhesive, optical)
  - ✓ 0.1 mL flat PCR tube 8-cap strips (optical)
- Nucleic acid extraction Kit
  - ✓ RADI Prep Swab and Stool DNA/RNA Kit
- Viral Transport Medium
  - ✓ Clinical Virus Transport Medium
- Cold block
- Vortex mixer
- PCR Tube rack

5. Storage

- All components should be stored at -25℃ to -15℃ upon arrival.
- Exposure time at Room temperature (25℃) should not exceed 30 minutes after the component being mixed.
- Freeze-thaw cycles should not exceed 5 times.

6. Specimen collection, transportation and storage

- The RADI Monkeypox Detection KIT is a standard specimen collection obtained from human skin lesion, crust or swab.
- All clinical specimens should be transported in non-breakable transport containers to eliminate the possibility of infection through leakage. In addition, when transporting to another area, the relevant country's biohazard transport guidelines must be followed.
- Specimens types of skin lesion, crust or swab collection tube is stable up to 7days at 2℃ to 8℃.
- Specimen collection device manufacturer's protocol and instructions for sample collection must be followed while skin lesion, crust or swab.
  Reference: Microbiology Specimen Collection and Transport
  https://www.healthcare.uiowa.edu/path_handbook/appendix/micro/micro_spec_collection.htm

7. Extraction

Samples are extracted to collect the nucleic acids using manual reagent RADI Prep Swab and Stool DNA/RNA Kit etc.

1) Freshly collected specimens should be used to collect DNA to ensure suitable DNA quality and quantity.
2) Users should prepare the positive control (PC) and no template control (*NTC), simultaneously alongside the specimen.

*NTC: RNase free water is supplied as a Kit component.

3) 200 ㎕ of specimen sample to be extracted should be matched with the manufacturer's instruction for the volume of specimen required for extraction.
4) RADI Prep Swab and Stool DNA/RNA Kit is recommended to extract the DNA from the freshly collected specimen and must follow the manufacturer's instructions.

5) The extracted DNA should be used immediately or stored at -70°C for later use.
6) Precautions should be taken while handling the positive control to avoid cross-contamination of other samples in the test run.
7) False positive results may appear due to the failure of taking proper precautions while handling the positive control.

Note.

- The extraction protocol or extraction Kit's quality may affect real-time PCR results.
- The quality of the extracted DNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology.

8. PCR Mixture Protocol

All reagent should be mixed in the preparation area (PCR workstation or equivalent amplicon-free area).

1) As the Kit (reagents) is stored in a frozen condition, take the Kit out and thaw thoroughly at ambient temperature.
2) Vortex and centrifuge each reagent briefly. The reagents should be kept on ice at all times of the experiments.
3) Calculate the number of reactions (n) that will be included in the test.
4) The number of the reactions should include NTC and Positive control (1 tube each) and each specimen to be tested.
5) Calculate the concentration of "2X RADI FAST MasterMix" and "Monkeypox Primer & Probe Mixture" needed for desired number of reactions and aliquot them into a 1.5 ml tube and vortex to mix well.

Reagent and ingredient concentration for PCR Mixture preparation:

Component		Volume (μℓ)	Volume (μℓ) n reaction
PCR Mixture	2X RADI FAST MasterMix	10	10 x number of reaction (n)
	Monkeypox Primer & Probe Mixture	5	5 x number of reaction (n)
Extracted DNA, PC, NTC		5	5 X number of reaction (n)
Total Volume		20	20 x number of reaction (n)

6) Prepare 96-well plates or tube strips for real-time PCR based on the estimated number of reactions (n).
7) Aliquot 15 μℓ of PCR-Mix into each well.
8) Add 5 μℓ of extracted DNA (specimen) into each well and note the specimen ID and well position (table above).
9) For negative well, add 5 μℓ of RNase free water (table above).
10) For positive well, add 5 μℓ of Monkeypox Positive Control (table above).
11) Cover the plate or tubes, vortex and spin down then transfer them into PCR area for PCR run.
12) The remaining Reaction Mix and reagents must be stored at -20°C.

Note.

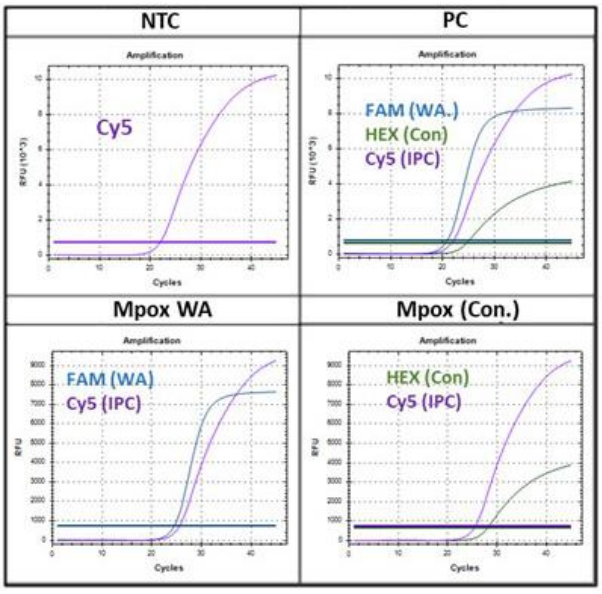
- Considering the pipette loss, users may add one or more reaction PCR mixture to the calculated quantity.
- To avoid contamination from the PC, pipette the positive control at the last.
- Must not change the volumes for reagent preparation specified in table above or the volume of the sample. Such changes could cause false results.

9. PCR amplification Protocol

Reference Images after Threshold adjusted amplification curve graphs from CFX96

Temperature	Time	Cycle
95 ℃	20 sec	1
95 ℃	2 sec	45
60* ℃	5 sec	

* Fluorogenic data should be collected during this step through the FAM, HEX, and Cy5 channels.



10. Quality Control

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results are also invalid. A negative control and a positive control should be set for each batch.

Control	West African (FAM)	Congo Basin (HEX)	IPC (Cy5)
NTC	No Ct or Ct>40	No Ct or Ct>40	No Ct or Ct>40
PC	18≤Ct≤34	18≤Ct≤34	18≤Ct≤34

11. Test Interpretation

Test interpretation of RADI Monkeypox Detection KIT is described as below.

Ct value of Target genes	Result on Clinical specimens
≤40	Detected (+)
No Ct or > 40	Not detected (-)

Cases	Target			Result
	W. Africa (FAM)	Congo Basin (HEX)	IPC (Cy5)	
1	≤40	> 40 or —	≤40	W. A MP Positive
2	≤40	> 40 or —	—*	W. A MP Positive
3	> 40 or —	≤40	≤40	Congo B. MP Positive
4	> 40 or —	≤40	—*	Congo B. MP Positive
5	> 40 or —	> 40 or —	≤40	Negative
6	> 40 or —	> 40 or —	—	Invalid** (Re-testing)

* Whenever IPC Ct range within ≤40, the test is valid. In positive cases, IPC may not be detected due to competitive reaction with target genes.
** Results are invalid. Re-test is required.

12. Test Limitations

1) Performance of the Kit has been established in skin lesion, crust or swab specimens from symptomatic individuals suspected of Monkeypox.
2) This Kit is a qualitative test and does not provide the quantitative value.
3) 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.
4) Negative results do not preclude Monkeypox Virus and should not be used as the sole basis for treatment or other patient management decisions.
5) Detection of viral DNA may not indicate the presence of infectious bacteria or that bacteria is the causative agent for clinical symptoms.
6) False positive results may happen from cross-contamination between patient samples, specimen mix-up and/or DNA contamination during product handling.
7) False-negative results may arise from:
   - Improper sample collection
   - A sample at concentrations near or below the limit of detection of the test.
   - Degradation of the viral DNA during specimen transport and/or storage.
   - Failure to follow the Instructions for Use provided

13. Performance characteristics

Analytical sensitivity (Limit of Detection)

In order to determine limit of detection (LOD) of RADI Monkeypox Detection KIT, a tentative LOD is established with several diluted concentrations using synthesized DNA. After a tentative LOD is established, the claimed LOD was established with 20 replicates of diluted concentrations spanning tentative LOD.

- **Claimed LoD**: 10 copies/μL

Target	Copies/μL
West African	10.00
Congo Basin	10.00

- **Cut off Value**

A Ct value of 40 was set as the cut-off of RADI Monkeypox Detection KIT.

Cross Reactivity

RADI Monkeypox Detection KIT did not cross-react with any of 21. IPC was all detected 20~35 Ct range.

No.	Reference	Cat. No.	Pathogen	Result
0-1			NTC	Not detected
0-2			Positive control	Target detected
1	NCCP	15882	*Streptococcus pneumoniae*	Not detected
2	NCCP	72002	*Legionella pneumophila*	Not detected
3	NCCP	72026	*Bordetella pertussis*	Not detected
4	NCCP	72006	*Pseudomonas aeruginosa*	Not detected
5	NCCP	72077	*MycobaCterium tuberculosis*	Not detected
6	ATCC	29342DQ	*Mycoplasma pneumoniae*	Not detected
7	NCCP	43193	Adenovirus	Not detected
8	NCCP	43252	Dengue virus serotype 1	Not detected
9	NCCP	43248	Dengue virus serotype 2	Not detected
10	NCCP	43256	Dengue virus serotype 3	Not detected
11	NCCP	43257	Dengue virus serotype 4	Not detected
12	NCCP	43280	Zika virus	Not detected
13	Vircell	MBC100	Yellow Fever Virus	Not detected
14	NCCP	43230	Influenza A virus (H3N2)	Not detected
15	NCCP	43231	Influenza A virus (H1N1)	Not detected
16	NCCP	43232	Influenza B virus (Yamagata)	Not detected
17	NCCP	43238	Respiratory Syncytial virus A	Not detected
18	NCCP	43239	Respiratory Syncytial virus B	Not detected
19	NCCP	43214	human Coronavirus NL63	Not detected
20	NCCP	43261	SFTS virus	Not detected
21	NCCP	43326	SARS-CoV-2	Not detected

Interfering test

RADI Monkeypox Detection KIT does not have any interference with following interfering substances.

No.	Interfering Substances	Concentrations	Interference
1	Bilirubin	30 mg/dL	No interference
2	Hemoglobin	2 g/dL	No interference
3	Triglyceride	1 g/dL	No interference

14. Troubleshooting

Please contact to info@khmedical.co.kr for Troubleshooting guide.

15. Bibliography

1.Monkeypox, key facts. https://www.who.int/news-room/fact-sheets/detail/monkeypox
2.Monkeypox: diagnostic testing. https://www.gov.uk/guidance/monkeypox-diagnostic-testing
3.Karem, Kevin L et al. "characterization of acute-phase humoral immunity to monkeypox: use of immunoglobulin M enzyme-linked immunosorbent assay for detection of monkeypox infection during the 2003 North American outbreak." Clinical and diagnostic laboratory immunology

vol. 12,7 (2005): 867-72. doi:10.1128/CDLI.12.7.867-872.2005
4.Li, Daniel et al. "Evaluation of the GeneXpert for Human Monkeypox Diagnosis." The American journal of tropical medicine and hygiene vol. 96,2 (2017): 405-410. doi:10.4269/ajtmh.16-0567

16. Description of Symbol Used

Symbol	Description	Symbol	Description
REF	Catalogue number		Consult instruction for use
LOT	Lot number	-25℃ -15℃	Storage at -25℃ to -15℃
	Use by date		Manufacturer
Σ	Contains sufficient for tests	CE	CE mark
IVD	In vitro diagnostic Medical Device	EC REP	Authorized representative in the European community

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CE IVD