

VIASURE

Real Time PCR Reagents RUO



VIASURE Monkeypox virus Real Time PCR Reagents RUO for BD MAX™ System

RUO

For Research Use Only (RUO). Not for use in diagnostic procedures.

This product has no declared clinical intended purpose and is not for clinical diagnostic use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.



These instructions apply to the following reference:

PRODUCT	REFERENCE
VIASURE <i>Monkeypox virus</i> Real Time PCR Reagents RUO for BD MAX™ System	VS-MPX224RUO 444211

Table A 1. Reference for product to be used with the BD MAX™ System.

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ENGLISH

1. Use

For Research Use Only (RUO). Not for use in diagnostic procedures.

VIASURE Monkeypox virus Real Time PCR Reagents RUO for BD MAX™ System is a real-time PCR test designed for the qualitative identification of DNA from Monkeypox virus nucleic acid. This test must be used for research purposes and has no medical objective. DNA is extracted from research samples, amplified using real-time PCR and detected using fluorescent reporter dye probes specific for Monkeypox virus.

2. Summary and Explanation

Monkeypox virus (MPXV) is an enveloped double-stranded DNA virus. It belongs to the *Orthopoxvirus* genus of the *Poxviridae* family. The *Orthopoxvirus* genus also includes Vaccinia virus, Cowpox virus, Variola virus and several other, animal-related, poxviruses. Two phylogenetically distinct clades of MPXV have been identified through genomic sequencing: the Central African (Congo Basin) clade and the West African clade.

3. Principle of the RUO kit

VIASURE Monkeypox virus Real Time PCR Reagents RUO for BD MAX™ System is designed for the identification of DNA from Monkeypox virus. After nucleic acid isolation, the detection of Monkeypox virus is performed by the amplification of a conserved region of G2R and F3L genes for Monkeypox virus using specific primers and fluorescent-labelled probes.

VIASURE Monkeypox virus Real Time PCR Reagents RUO for BD MAX™ System is based on the 5' exonuclease activity of DNA polymerase. During DNA amplification, this enzyme cleaves the probe bounded to the complementary DNA sequence, separating the quencher dye from the reporter. This reaction generates an increase in the fluorescent signal which is proportional to the quantity of target template. This fluorescence can be measured on Real Time PCR platforms.

VIASURE Monkeypox virus Real Time PCR Reagents RUO for BD MAX™ System contains in each well all the components necessary for real time PCR assay (specific primers/probes, dNTPs, buffer and polymerase) in a stabilized format, as well as an endogenous internal (EIC) control to monitor the extraction process and/or the inhibition of the polymerase activity.

Target	Channel	Gene
Monkeypox virus	475/520	G2R and F3L genes
Endogenous Internal control (EIC)	585/630	hemoglobin subunit beta gene

Table 1. Target, channel and genes.

4. Reagents provided

VIASURE *Monkeypox virus* Real Time PCR Reagents RUO for BD MAX™ System includes the following materials and reagents detailed in Table 2:

Reagent	Description	Barcode	Amount
<i>Monkeypox virus</i> reaction tube	A mix of enzymes, primers-probes, buffer, dNTPs, stabilizers and endogenous internal control in stabilized format	1H foil	2 pouches of 12 transparent tubes
Rehydration Buffer tube	Solution to reconstitute the stabilized product	11 foil	1 pouch of 24 transparent tubes

Table 2. Reagents and materials provided in VIASURE *Monkeypox virus* Real Time PCR Reagents RUO for BD MAX™ System with Cat. N°. 444211.

5. Reagents and equipment to be supplied by the user

The following list includes the materials and equipment that are required but not included in the VIASURE *Monkeypox virus* Real Time PCR Reagents RUO for BD MAX™ System.

- Real-time PCR instrument: BD MAX™ System (Ref: 441916).
- BD MAX™ ExK™ TNA-3 (Ref:442827 or 442828).
- BD MAX™ PCR Cartridges (Ref: 437519).
- Vortex.
- Micropipettes (accurate between 2 and 1000 µL).
- Nuclease-free water.
- Filter tips.
- Powder-free disposable gloves.

6. Transport and storage conditions

- The RUO kits can be shipped and stored at 2-40°C until the expiration date which is stated on the label.
- After opening the aluminum pouches which contain the reaction tubes, the product should be used within 28 days.

7. Additional information

- VIASURE *Monkeypox virus* Real Time PCR Reagents RUO for BD MAX™ System is for Research Use Only. It is not for use in diagnostic or therapeutic procedures. The performance characteristics of this product have not been established.
- Do not remove desiccant from reagent pouches.
- Close protective pouches of reagents promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Do not use reagents if the foil has been broken or damaged.
- Protect reagents from humidity. Prolonged exposure to humidity may affect product performance.

- Keep components away from light.
- In cases where other PCR tests are conducted in the same general area of the laboratory, care must be taken to ensure that the VIASURE *Monkeypox virus* Real Time PCR Reagents RUO for BD MAX™ System, BD MAX™ ExK™ TNA-3 extraction kit, or any additional reagents required for testing and the BD MAX™ System are not contaminated. Always avoid microbial and ribonuclease (RNase)/deoxyribonuclease (DNase) contamination of reagents. The use of sterile RNase/DNase-free disposable aerosol resistant or positive displacement pipette tips is recommended. Use a new tip for each sample. Gloves must be changed before manipulating reagents and cartridges (BD MAX™ PCR Cartridge).
- To avoid contamination of the environment by amplicons, do not break apart the BD MAX™ PCR Cartridge after use. The seals of the BD MAX™ PCR Cartridge are designed to prevent contamination.
- Design a unidirectional workflow. It should begin in the Extraction Area and then move to the Amplification and Detection Area. Do not return samples, equipment, and reagents to the area in which the previous step was performed.
- Follow Good Laboratory Practices. Wear protective clothing, use disposable gloves, goggles, and mask. Do not eat, drink, smoke or apply cosmetic products in the working area. Wash your hands after finishing the test.
- Samples must be treated as potentially infectious and/or biohazardous, as well as all the reagents and materials that have been exposed to the samples and they must be handled according to the national safety regulations. Take necessary precautions during the collection, transport, storage, handling, and disposal of samples.
- Samples and reagents must be handled in a biological safety cabinet. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples. Dispose of waste in compliance with local and state regulations.
- Regular decontamination of commonly used equipment is recommended, especially micropipettes and work surfaces.
- In accordance with Regulation (EC) No 1907/2006 (REACH), VIASURE Real Time PCR Kits do not require Safety Data Sheets on account of their classification as non-hazardous to health and the environment, because they do not contain substances and/or mixtures which meet the hazard classification criteria available in Regulation (EC) No 1272/2008 (CLP), or which are in concentrations higher than the value established in the mentioned regulation for their declaration.
- Consult the BD MAX™ System User's Manual for additional warnings, precautions and procedures.

8. Test procedure

8.1. Sample collection, storage and transport

Collection, storage, and transport of samples should be maintained per the conditions validated by the user and according to appropriate laboratory guidelines.

8.2. Sample preparation and DNA extraction

Perform the sample preparation according to the recommendations in the instructions of extraction kit used, BD MAX™ ExK™ TNA-3.

1. Pipette 200-400 µL of sample into a BD MAX™ ExK™ TNA-3 Sample Buffer Tube and close the tube with a septum cap. Ensure complete mixing by vortexing the sample at high speed for 1 minute. Proceed to BD MAX™ System Operation. If the internal control fails, repeat the DNA extraction reducing the sample volume.

Note: The volume of sample pipetted into the Sample Buffer Tube must be validated by the user according to the type of sample used.

8.3. PCR protocol

Note: Please, refer to the BD MAX™ System User's Manual for detailed instructions.

8.3.1. Creating PCR test program for VIASURE Monkeypox virus Real Time PCR Reagents RUO for BD MAX™ System

Note: If you have already created the test for the VIASURE Monkeypox virus Real Time PCR Reagents RUO for BD MAX™ System, you can skip step 8.3.1 and go directly to 8.3.2.

- 1) On the "Run" screen of the BD MAX™ System, select the "Test Editor" tab.
- 2) Click the "Create" button.
- 3) In the Basic Information tab, within the "Test Name" window, name your test: i.e. VIASURE Monkeypox RUO.
- 4) In the "Extraction Type" drop down menu, select "ExK TNA-3".
- 5) In the "Master Mix Format" drop down menu, choose "Type 5".
- 6) In the "Sample extraction parameters" select "User defined" and adjust sample volume setting to 350-950 µL.

Note: The sample volume must be validated and adjusted by the user according to the type of sample used.

- 7) In the "Ct Calculation" select "Call Ct at Threshold Crossing".
- 8) If running software version 5.00 or higher and have barcoded foil snap-in tubes, in the "Custom Barcodes" select the following configuration:
 - a. Snap-In 2 Barcode: 1H (concerning Monkeypox virus reaction tube).
 - b. Snap-In 3 Barcode: 11 (concerning Rehydration Buffer tube).
- 9) In "PCR settings" tab enter the following parameters: "Channel Settings", "Gains" and "Threshold" (Table 3).
 - a. Note: It is recommended to set the minimum threshold values listed below for each channel as a starting point, but the final settings must be determined by the end-user during the result interpretation, in order to ensure that thresholds fall within the exponential phase of the fluorescence curves and above any background signal.

Channel	Alias	Gain	Threshold	Ct Min	Ct Max
475/520 (FAM)	MPXV	80	200	0	40
530/565 (HEX)	-	-	-	-	-
585/630 (ROX)	IC	80	200	0	35
630/665 (Cy5)	-	-	-	-	-
680/715 (Cy5.5)	-	-	-	-	-

Table 3. PCR settings.

10) In "PCR settings" tab enter the following parameters "Spectral Cross Talk" (Table 4), as well.

		False Receiving Channel					
		Channel	475/520	530/565	585/630	630/665	680/715
Excitation Channel	475/520	-	0.0	0.0	0.0	0.0	0.0
	530/565	0.0	-	0.0	0.0	0.0	0.0
	585/630	0.0	0.0	-	0.0	0.0	0.0
	630/665	0.0	0.0	0.0	-	0.0	0.0
	680/715	0.0	0.0	0.0	0.0	-	0.0

Table 4. Spectral cross-talk parameters.

11) In "Test Steps" tab, enter the PCR protocol (Table 5).

Step Name	Profile Type	Cycles	Time (s)	Temperature	Detect
Initial denaturation	Hold	1	120	98°C	-
Denaturation and Annealing/Extension (Data collection)	2-Temperature	45	10	95°C	-
			58	60°C	✓

Table 5. PCR protocol.

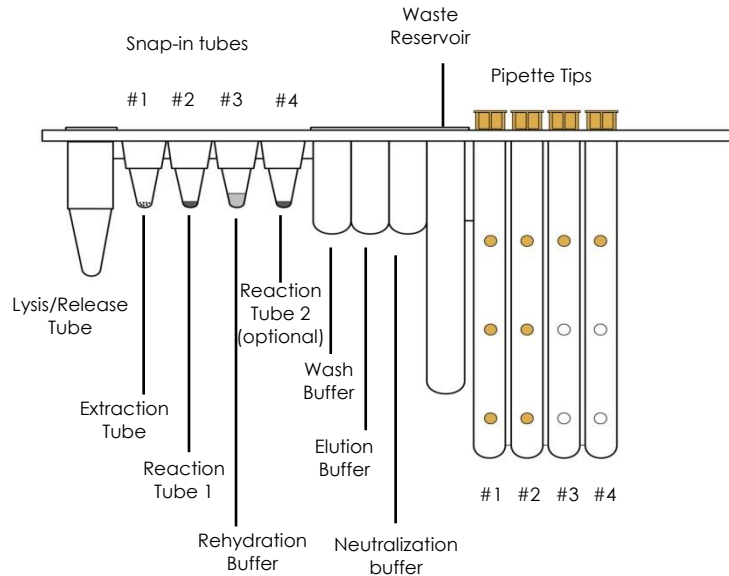
12) Click the "Save Test" button.

8.3.2. BD MAX™ Rack set up

- For each sample to be tested, remove one Unitized Reagent Strips from the BD MAX™ ExK™ TNA-3 kit. Gently tap each strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes and load on the BD MAX™ System sample racks.
- Remove the required number of BD MAX™ ExK™ TNA Extraction Tubes (B4) (white foil) from their protective pouch. Snap the Extraction Tube(s) (white foil) into its corresponding positions in the TNA strip (Snap position 1, white color coding on the rack. See Figure 1). Remove excess air, and close pouch with the zip seal.
- Determine and separate the appropriate number of *Monkeypox virus* reaction tubes (1H foil) and snap into their corresponding positions in the strip (Snap position 2, green color coding on the rack. See Figure 1).
 - Remove excess air, and close aluminum pouches with the zip seal.
 - In order to carry out a correct rehydration, please make sure that the lyophilized product is in the bottom of the tube and is not adhered to the top area of the tube or to the foil seal. Gently tap each tube on a hard surface to make sure all the product is at the bottom of the tube.

- 4) Remove the required number of Rehydration Buffer tubes (11 foil) and snap into their corresponding positions in the strip (Snap position 3, non-color coding on the rack. See Figure 1). Remove excess air and close the pouch with the zip seal.
 - a. In order to ensure a correct transfer, please make sure that the liquid is in the bottom of the tube and is not adhered to the top area of the tube or to the foil seal. Gently tap each tube on a hard surface to make sure all the buffer is at the bottom of the tube.

Figure 1. BD MAX™ TNA Reagent Strip (TNA) from the BD MAX™ ExK™ TNA-3 kit.



8.3.3. BD MAX™ Instrument set up

- 1) Select the "Work List" tab on the "Run" screen of the BD MAX™ System software v4.50A or higher.
- 2) In the "Test" drop down menu, select *VIASURE Monkeypox virus RUO* (if not already created see Section 8.3.1).
- 3) Select the appropriate kit lot number (found on the outer box of extraction kit used) from the pull-down menu (optional).
- 4) Enter the Sample Buffer Tube identification number into the Sample tube window of the Work List, either by scanning the barcode with the scanner or by manual entry.
- 5) Fill the ID and/or Accession window of the Work List and click the "Save" button. Continue until all Sample Buffer Tubes are entered. Ensure that the specimen and the Sample Buffer Tubes are accurately matched.
- 6) Place the prepared Sample Buffer Tube into the BD MAX™ Rack(s).
- 7) Load the rack(s) into the BD MAX™ System (Rack A is positioned on the left side of the BD MAX™ System and Rack B on the right side).
- 8) Place the required number of BD MAX™ PCR Cartridge(s) into the BD MAX™ System.
- 9) Close the BD MAX™ System door.
- 10) Click "Start Run" to begin the procedure.

8.3.4. BD MAX™ report

- 1) In main menu, click the "Results" button.
- 2) Either double click on your run in the list or press the "view button".
- 3) Click on "Print", select: "Run Details, Test Details and Plot...".
- 4) Click on "Print or Export button" on the "Run Reports" screen.

9. Result interpretation

For research use only. Not for use in diagnostic procedures.

The analysis of the data is done by the BD MAX™ software according to the manufacturer's instructions. The BD MAX™ software reports Ct values and amplification curves for each detector channel of each sample tested in the following way:

- Ct value of 0 indicates that there was no Ct value calculated by the software with the specified Threshold (see Table 3). Amplification curve of the sample showing a "0" Ct value must be checked manually.
- Ct value of -1 indicates that no amplification process has occurred.
- Any other Ct value should be interpreted in correlation with the amplification curve.

Check Internal Control signal to verify the correct functioning of the amplification mix.

-A sample is considered negative, if the sample shows no amplification signal in the detection system but the internal control is positive ($Ct < 35$).

-A sample is considered positive if the Ct value obtained is less than 40. The endogenous Internal Control (IC) may or may not show an amplification signal. Sometimes, the IC detection is not necessary because a high copy number of the target can cause preferential amplification of target-specific nucleic acids.

An inhibition of the PCR reaction can be excluded by the amplification of internal control. An unresolved result (UNR) is the absence of both target and internal control amplification signals.

An Indeterminate assay result (IND) is reported due to a BD MAX™ System failure in case of an instrument failure linked to an error code.

An Incomplete assay result (INC) is reported due to a BD MAX™ System failure in case of failure to complete run.

10. Limitations of the test

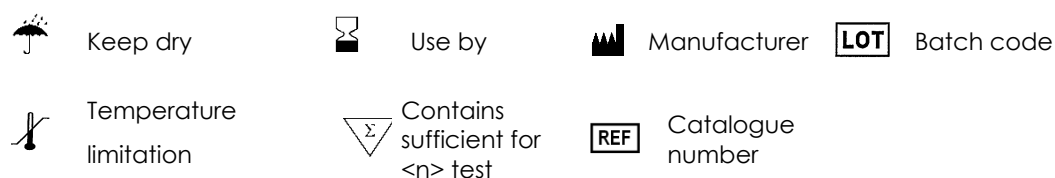
- The test is to be used for research use only. Not for use in diagnostic procedures.

- For good test performance, the lyophilized product should be at the bottom of the tube and not adhered to the top area of the tube or the foil seal. Gently tap each tube on a hard surface to make sure all the product is at the bottom of the tube.
- An appearance of the reaction mixture in stabilized format, normally found at the bottom of the tube, different from the usual one (without conical shape, inhomogeneous, smaller/larger in size and/or color different from whitish) does not alter the functionality of the test.
- This RUO test is a qualitative test and does not provide quantitative values or indicate the number of organisms present.
- Extremely low levels of target below the limit of detection might be detected, but results may not be reproducible.
- In the case of obtaining Unresolved, Indeterminate or Incomplete results using VIASURE Monkeypox virus Real Time PCR Reagents RUO for BD MAX™ System, retesting can be performed. Unresolved results may be due to the presence of inhibitors in the sample or an incorrect rehydration of lyophilized reaction mix tube. If there is an instrument failure, Indeterminate or Incomplete results will be obtained.

11. Quality control

VIASURE Monkeypox virus Real Time PCR Reagents RUO for BD MAX™ System contains an Internal Control (IC) in each reaction tube which confirms the correct performance of the technique.

Symbols for components and reagents



Trademarks

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Technical Service and Support: In the United States contact BD at 1.800.638.8663 or bd.com. For regions outside of the United States, contact your local BD representative or bd.com.

Change Control		
Version No.	Changes	Date
00	Original version	01/09/2022

Table A 2. Control change table.

Revision: 1st September 2022

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One step ahead



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