Instructions For Use

CE IVD *For In Vitro Diagnostic Use*

Store at -25°C to -15°C

REF **MP-RT50**

50

geneMAP™ Monkeypox PCR Detection Kit

For Real-Time PCR

Multiplex Real-time PCR System for detection of Monkeypox virus

2-Plex (FAM, VIC)

Validated on dual color insturments such as:

* Biorad® CFX96, Real-time PCR System (Bio-Rad)
* LC480, Roche
* Rotorgene Q5/Q6, Qiagen
* Mic/Biomolecular System
* ThermoScientific ABI7500 and QuantStudio Series

Contents

1. Intended Use

The geneMAP™ Monkeypox PCR Detection Kit is qualitative in vitro assay for the detection of Monkeypox virus from Nasopharyngeal swab, Oropharyngeal swab, Serum and Rash Fluid.

2. Safety Instructions & General Warnings

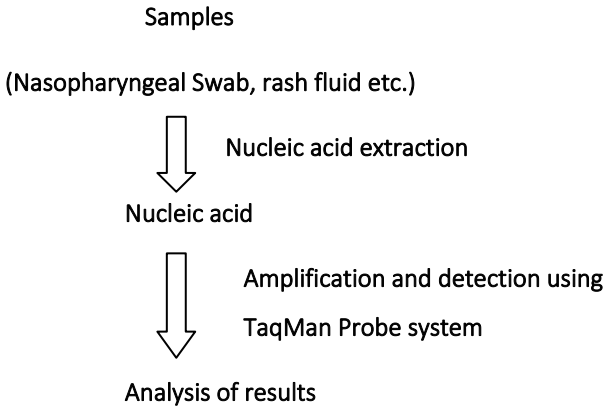
- This kit must be used strictly in accordance with the instructions provided in this manual, and only in combination with validated reagents and instruments. Any off-label use of this product, as well as any modification of its components, will nullify Genmark's liability.
- Obey proper laboratory safety protocols when working with chemicals and specimens.
- The protocol can be performed by only professional and trained personal.
- Perform the protocol in a well-ventilated and well-lit environment.
- Store the kit and its components within recommended temperature range in de-frost refrigerators. Do not use no-frost refrigerators.
- Avoid skin contact with any of the reagents in the kit
- Wipe workspace surfaces with 10% bleach followed by 70% alcohol.
- Instruments may exhibit performance variations due to differences in electrical currents and power outlets, as well as the effects of maintenance and calibration. It is the responsibility of the user to ensure that all instruments are properly maintained and regularly calibrated according to the manufacturer's guidelines. Genmark disclaims responsibility for any performance issues arising from improper maintenance, calibration, or variations in electrical supply.
- All biological specimens should be handled as potentially infectious, following standard precautions. For guidelines on specimen handling, refer to the World Health Organization.
- Consult environmental waste personnel for proper disposal of used plates, consumables, and reagents, considering federal and local hazardous waste regulations. Check local and national disposal requirements.

3. Principles and Procedure Overview

3.1 Principles

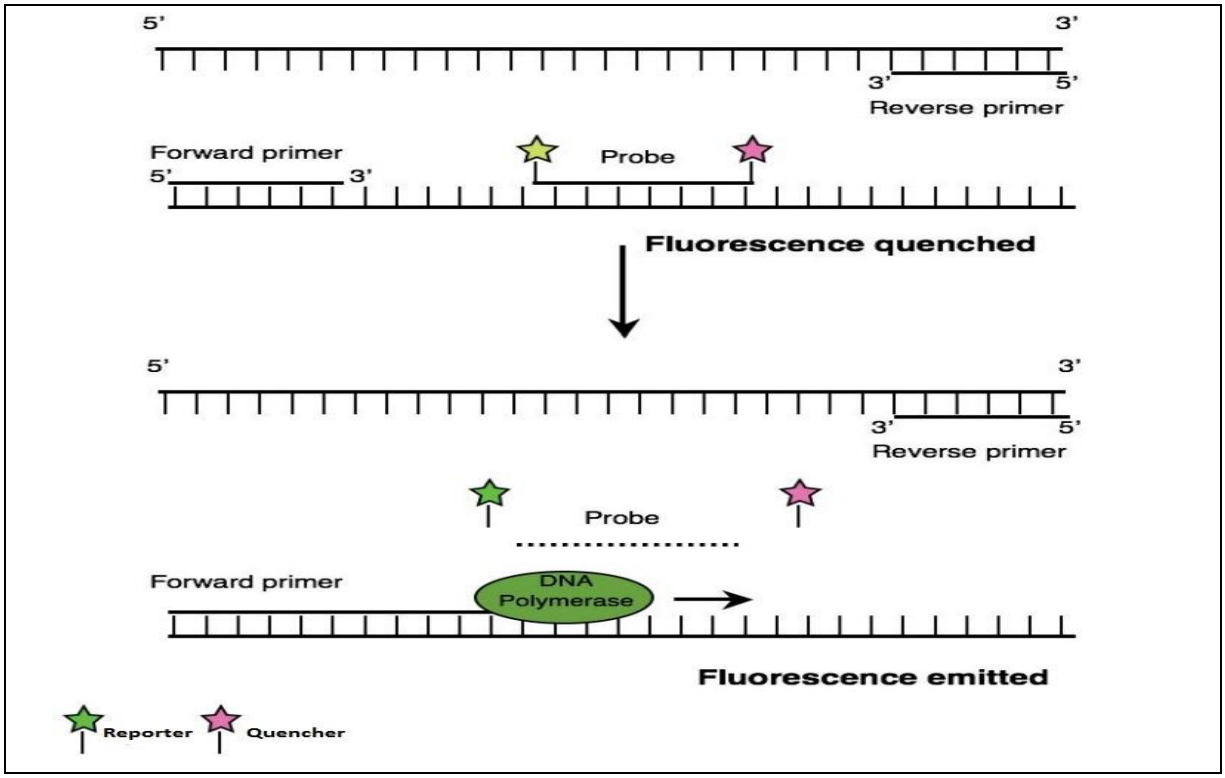
The polymerase chain reaction (PCR) is sensitive and specific TaqMan Probe technology with the use of DNA amplification technique, primer design and PCR optimization. The kit is, based on two main processes: nucleic acid extraction and PCR amplification of nucleic acid in the primer and probe mechanism of PCR machines by Real-time PCR. The kit is a real-time PCR test where Monkeypox and Internal Control (IC) target is a multiplex realization that allows amplification of nucleic acids.

Procedure Overview;

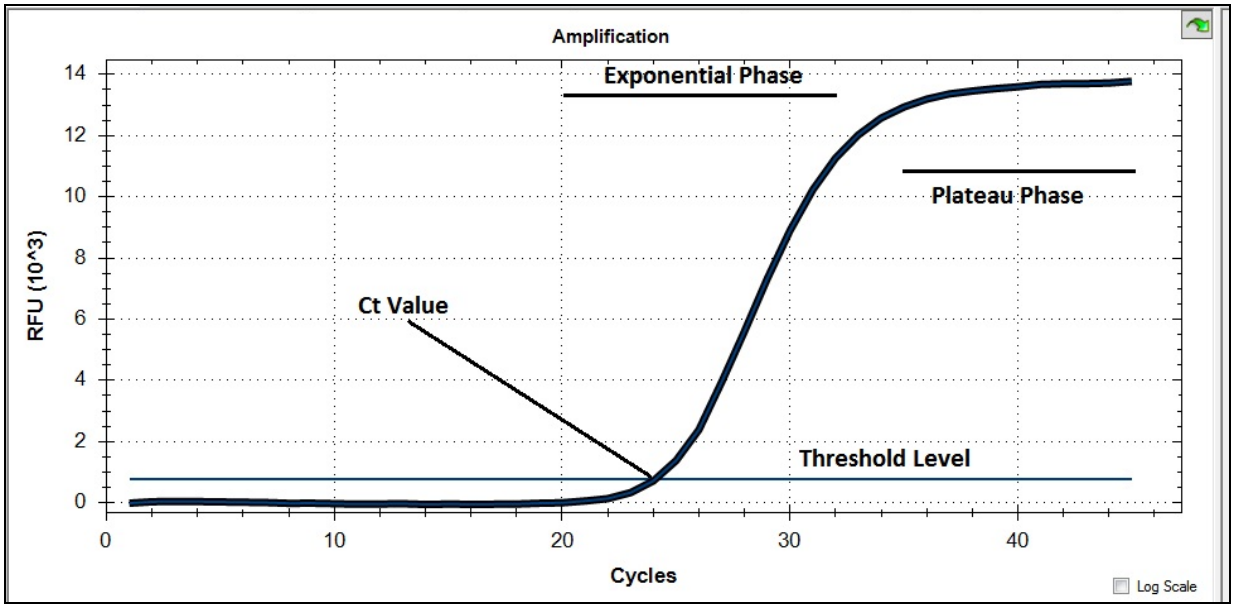


3.2 Technology

Hydrolysis (TaqMan) probes are the most common form of qPCR probes and are widely used in human, veterinary, and environmental diagnostics. These probes utilize a fluorescent dye at one end of the DNA oligonucleotide and a quencher at the other. During PCR, the probe specifically anneals to the target DNA sequence (from sample), which is flanked by the two primers. As DNA polymerase extends the new DNA strand, the probe is degraded by the 5' to 3' exonuclease activity of the polymerase, resulting in the fluorophore being separated from the quencher and emitting fluorescence. The more DNA present in the reaction, the earlier the fluorescence reaches a detectable level resulting in earlier Ct values.



Hydrolysis (TaqMan) Probe Technology.



Typical Amplification Plot of Real-time PCR in Linear Scale Graphic.

4. Background Information

Monkeypox is a rare disease caused by infection with the monkeypox virus. Monkeypox virus belongs to the Orthopoxvirus genus in the family Poxviridae. The Orthopoxvirus genus also includes variola virus (which causes smallpox), vaccinia virus (used in the smallpox vaccine), and cowpox virus. Monkeypox is not related to chickenpox.

Monkeypox was first discovered in 1958 when two outbreaks of a pox-like disease occurred in colonies of monkeys kept for research. Despite being named "monkeypox", the source of the disease remains unknown. However, African rodents and non-human primates (like monkeys) may harbor the virus and infect people.

The first human case of monkeypox was recorded in 1970. Since then, monkeypox has been reported in people in several other central and western African countries. Prior to the 2022 outbreak, nearly all monkeypox cases in people outside of Africa were linked to international travel to countries where the disease commonly occurs, or through imported animals. (1)

5. Reagents

Reagents contained in a kit are sufficient for 50 reactions.

Description	No. of Reactions	No. of Tubes	Vol. Per Tube	Color of Caps	Description
2X Master Mix with UDG	50	1	550 µl	Clear	• DNA polymerase, UDG • Buffer containing dNTPs
4X Monkeypox Primer Probe Mix	50	1	275 µl	Amber	Primer Probe Mix (PPM): • Amplification and detection primer probe sets
Monkeypox Positive Control	5	1	100 µl	Red	Positive Control (PC): •Mixture of pathogen and IC clones
RNase Free Water	5	1	400 µl	Violet	RNase Free Water for Negative Template Control

*Note: Do not subject the tubes to more than 5 freeze-thaw cycles.

6. Storage and Handling

All components of the kit must be stored at between -15°C /-25°C. All components are stable under the recommended storage conditions until the expiration date indicated on the label on the box. The performance of the kit components are not affected until 5 freeze and thaw. If reagents are to be used only intermittently, they should be stored in aliquots.

This product is shipped on frozen blue ice packs (+4 °C) and may thawed upon arrival.

7. Materials Required But Not Provided

- Disposable powder-free gloves (latex or nitrile)
- Pipettes (adjustable) and sterile pipette tips
- 1.5 mL microcentrifuge tubes
- Desktop centrifuge
- Vortex mixer
- Clean bench
- For Biorad CFX Instruments;
    - 96-Well Skirted PCR Plate, White well (Cat. No. HSP-9655, Biorad)
    - Permanent Clear Heat Seal (Cat. No. 1814035, Biorad)

For the other instruments please use 96 well plates and tubes recommended by device manufacturers.

8. Protocol

8.1 Specimen Collection, Storage, and Transport

All samples should be considered as potentially infectious material. Only sample materials collected, stored and transported in accordance with the following rules and instructions are permitted.

To ensure a high sample quality, samples should be transported as quickly as possible. The samples should be transported at the specified temperatures.

8.1.1 Specimen Collection

Rash fluid

Select a fresh rash that is typical or transparent and clear, and after cleaning the surface twice using a sterile cotton swab with normal saline and drying the skin dry, puncture the rash with a sterile needle tip, squeeze out and dip the rash liquid with a sterile cotton swab and place it in the preservation solution immediately, and tighten the screw cap. Wipe the needle insertion site again with normal saline or disinfected alcohol, and press with a sterile dry cotton swab for a while to prevent infection.

Nasopharyngeal swab

Use a swab with the tip of polyester fiber to enter from the anterior nostril, and slowly go deep backward along the bottom of the inferior meatus. When the top of the swab reaches the posterior wall of the nasopharyngeal cavity (feeling against the wall), place the swab still for a moment (about 3 seconds), then gently rotate it for a round, slowly remove the swab, and put it into the tube. Break the plastic handle by hand, immerse the swab in the sampling solution, and tighten the tube cap. It has been proved that nasal swab samples can be preserved in preservation solutions such as saline or Hank's solution.

Throat swab

Use polyester fiber head swab to wipe the posterior uvula, posterior pharyngeal wall and bilateral tonsils repeatedly with moderate force and avoid touching the tongue. Take out the rear sampling tube, break the plastic handle on the contact part of the hand, soak the swab in the sampling solution, and tighten the tube cap. It has been proved that throat swab samples can be preserved in preservation solutions such as saline or Hank's solution.

Serum

Use a sterile syringe to collect 3-5mL of venous blood from the subject in a vacuum blood collection tube (preferably with separating gel), place at room temperature for no more than 4 hours, centrifuge at 3000rpm for 10 minutes, suck the upper serum (do not suck the red blood cells) and transfer to another sterile centrifuge tube for later use.

8.1.2 Specimen Storage and Transport

Specimen	Storage*		Transport**	Note
	Temp.	Duration	Temp.	
Nasopharyngeal swab	2-8°C	3 days	2-8°C	Store any leftover specimens at ≤-20°C
Rash Fluid & Serum				
Oropharyngeal swab				

*: Performance may be affected by routine freezing or prolonged storage of specimens.

**: Specimens should also adhere to local and national instructions for transport of pathogenic material.

8.2 Nucleic Acid Extraction

Various manufacturers offer nucleic acid extraction kits. Use the correct protocol according to the manufacturers' protocol. The following extraction kits have been validated for use with this kit.

8.2.1 Manual Nucleic Acid Extraction Kits

* Please use the recommended volumes of specimen and elution as indicated below. For all others, refer to the manufacturer's protocol.

Extraction Kit	Manufacturer	Cat. No	Recommended Vol.
QIAamp® MinElute® Virus Spin Kit*	QIAGEN	57704	Specimen:190µL Elution:40 µL
Ribo_spinvRD* (Viral RNA/DNA Extraction Kit)	GeneAll	302-150 SG1701	Specimen:290 µL Elution:40 µL

8.3 Preparation for Real-time PCR

* The correct tubes and caps must be used (see MATERIALS REQUIRED BUT NOT PROVIDED).
* Aerosol resistant filter tips and tight gloves must be used when preparing one-step RT-PCR reactions. Use extreme care to ensure no cross-contamination.
* Completely thaw all reagents on ice.
* Set up all reactions on ice to minimize the risk of RNA degradation.
* Briefly centrifuge the reagent tubes to remove drops from the inside of the cap.

2X Master Mix with UDG	10 µl
4X Primer Probe Mix	5 µl
Total volume of PCR Master Mix	15µl

* Calculate the necessary amount of each reagent needs based on the number of reactions (samples + controls).

1. Mix by inverting the tube 5 times or quick vortex, and centrifuge briefly.
2. Aliquot 15 µL of the one-step RT-PCR Master Mix into PCR tubes.
3. Add 5 µL of each sample's nucleic acids into the tube containing the one-step RT-PCR Master Mix.

PCR Master Mix	15 µl
Sample's nucleic acid	5 µl
Total volume of reaction	20 µl

* Use a new sterile pipette tip for each sample.
* For Negative Control (NC), use 5 µL of RNase-free Water instead of sample's nucleic acid.
* For Positive Control (PC), use 5 µL of PC.
* Please be careful not to cross-contaminate the one-step RT-PCR Master Mix and samples with the Positive Control.
* The PCR tubes must be mixed by pipetting and centrifuged before running PCR reaction. It needs to be checked that liquid containing all PCR components is at the bottom of each PCR tube.

9. Real-time PCR Instrument Setup and Results Analysis

9.1 Real-time PCR System

9.1.1 Pre-settings for Data Analysis

A. Pre-settings for Fluorophore Selection and Thermal Cycling Conditions

Target	Reporter Fluorophore
Monkeypox	FAM
Internal Control	VIC

For Biorad CFX96 (Duration 46 min.)

Temperature	Time	Cycles	Data Collection
25˚C	5 min	1	
95˚C	2 min	1	
95˚C	1 sec	44	
60˚C	1 sec		FAM, VIC

Note1: Please use only 72-well carousel for Qiagen Rotor-Gene® Q5/Q6, 36-well carousel is not recommended. Additionally, perform Auto-Gain optimization before first acquisition. (Auto-Gain optimization tube should be PC)

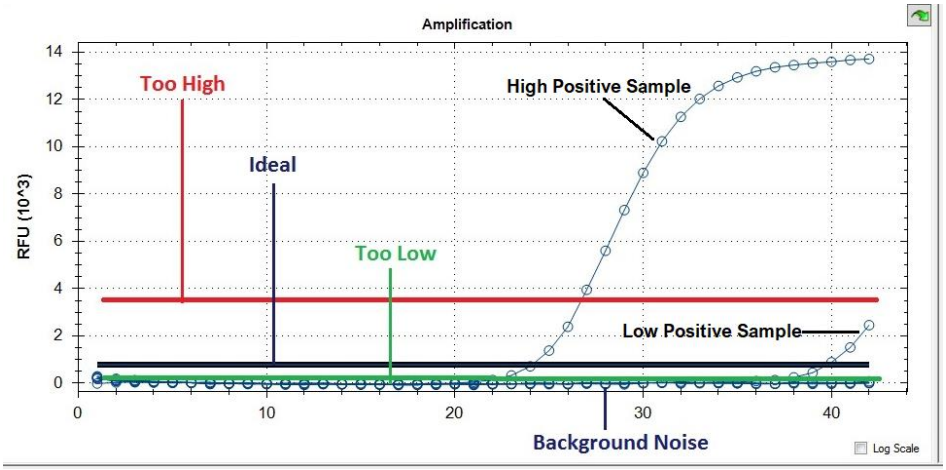
10. Results

10.1 General Rules of the Threshold Settings Manually

Normally the software-based methods will select a proper threshold, but in cases where the curves do not conform to the assumptions made by the algorithm, an incorrect threshold may be calculated. Good indicators of improperly-set threshold values are false positives (Ct values obtained from negative control wells), known positive samples giving very late Cts or no Cts at all. Because of those reasons manually adjusting the threshold is highly recommended.

Ideally, the threshold should be set in the region where the just above the background noise. The threshold should not be so high that it crosses any of the plots where they are starting to plateau and are no longer linear.

To adjust the threshold for each dye collected must be set separately.



Example of ideal threshold level.

If the threshold is too high, it gives false negative.

If the threshold is too low, it gives false positive.

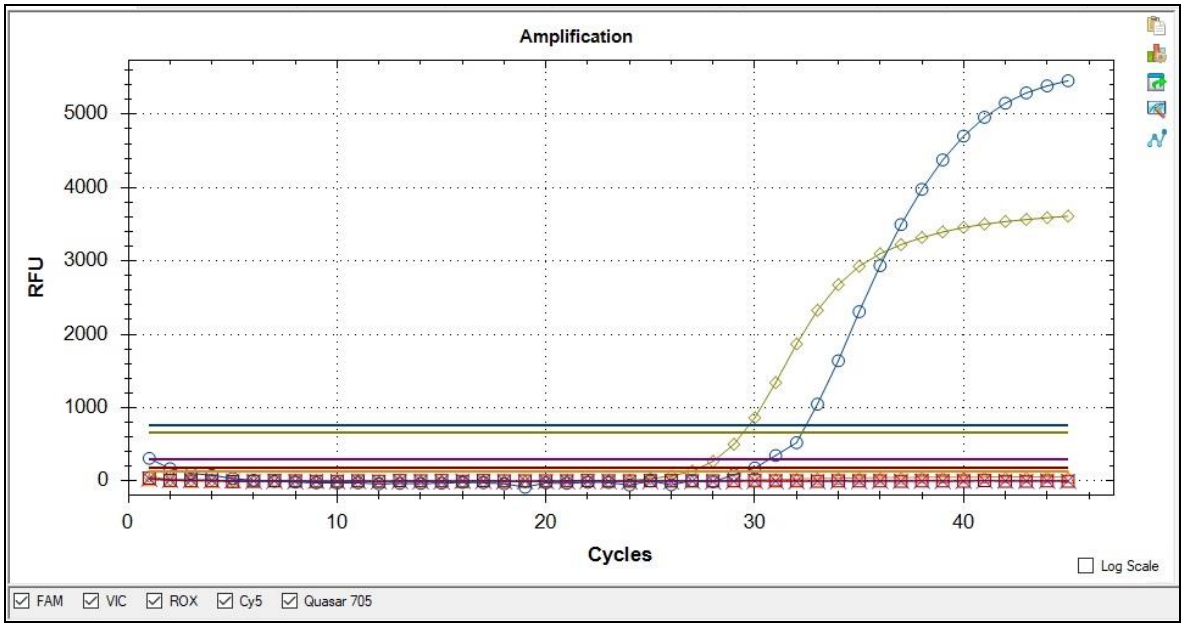
10.2 Interpretation of Results

Target	Reporter Fluorophore	Sample	
		Ct Value	Result
Monkeypox	FAM	<41	Positive (+)
		≥41 or NA	Negative (-)
Internal Control	VIC	<35	Positive (+)
		≥35 or NA	Negative (-)

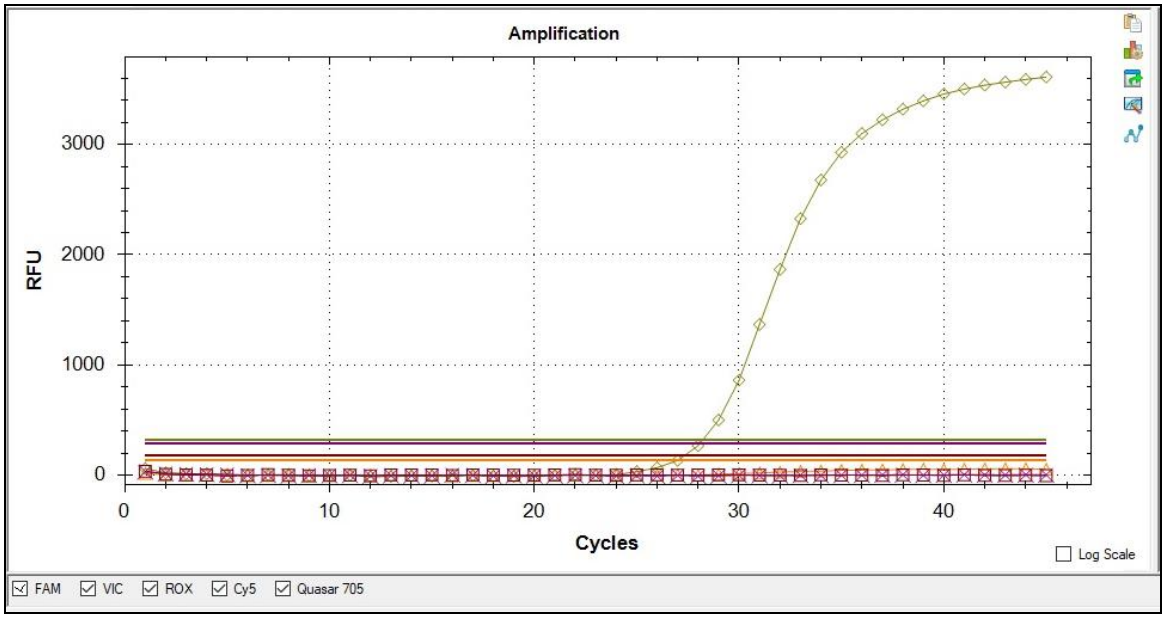
Interpretation	FAM	Internal Control	Reporting
Case 1	+	+/-	Monkeypox positive
Case 2	-	+	Monkeypox negative
Case 3	-	-	Invalid (Repeat the test)

* If amplification curve Ct is ≥36

** Repeat the test if the result is the same, confirm the result alternative tests



Monkeypox positive: FAM and VIC amplification curves are observed.



Monkeypox Negative: VIC (Internal Control) amplification curve are obsereved.

11. Performance Characteristics

11.1 Assay Specificity

11.1.1 *In Silico* Studies

In silico studies are summarized below;

No	Organism	In silico Analysis for % Identity target's
1	Monkeypox Virus	100% Match
2	Smallpox virus	No alignment found
3	Covpox virus	No alignment found
4	Herpes Simplex Virus 1	No alignment found
5	Herpes Simplex Virus 2	No alignment found

11.2 Assay Sensitivity

Limit of detection (LoD) of the kit is 300copies/mL

11.3 Reactivity/Inclusivity

An in silico inclusivity analysis of the geneMAP™ 's primers and probes was performed. All primer sets designed for detection of the targeted genes were tested against the complete available viruses genome sequence. The analysis demonstrated that the regions recognized by the designed primers and probes have 100% homology with all available target pathogen sequences from the National Center for Biotechnology Information (NCBI).

12. Limitations

Mutation in the target sequence of targeted pathogens or change in the sequence due to Virus Evolution can lead to false negative results.

False positive and false negative results can be caused by poor sample quality, incorrect sample collection, incorrect handling, incorrect laboratory processing, or restriction of testing technology.

13. Revision History

Date of Last Edit: August 2024		
Change	**Affected Section**	**Page**
Added reaction numbers for the kit	5.Reagents	6
Added ∑ (total numbers of tests) symbol	Cover Page	1
Added information related to Qiagen Rotor-Gene® Q5/Q6 set-up	9. Real-Time PCR Instrument Setup and Results Analysis	9
Updated Information	5. Reagents	6
Added Safety Instructions and General Warnings	2. Safety Instructions and General Warnings	3
Format updated	5. Reagents	6

14. References.

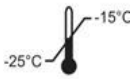
1- https://www.cdc.gov/poxvirus/monkeypox/about.html

15. Troubleshooting

geneMAP™ Monkeypox PCR Detection Kit		
OBSERVATION	POSSIBLE CAUSES	SOLUTION
No signal in any flororphore	Fluorophores incompatible with protocol for data analysis	Select the correct fluorophores.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect storage or past expiry date of the test kit	Please check the storage condition and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Presence of inhibitor	Repeat the test with the new extracted nucleic acid.
Amplification signals in Negative Control	Cross Contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Only use filter tips and throughout the procedure and change tips between tubes. Repeat entire procedure from nucleic acid extraction with the new set of reagents.
No amplification signal in Positive Control	The fluorophores for data analysis does not comply with the protocol	Please select the correct fluorophores for data analysis.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect PCR mixture	Confirm that all components are added to the PCR mixture. Sensitivity is compromised with pre-composed premix. All reagents must be homogenized and spun down before use.
	Did not add sample's nucleic acid	Please carefully repeat the test.
	Error in adding nucleic acid to corresponding PCR tubes	Check the sample numbers of tubes containing nucleic acid and make sure to add nucleic acid into the correct PCR tubes and carefully repeat the test if necessary.
	Incorrect storage or past expiry date of the test kit	Please check the storage condition (See page 6) and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Error in nucleic acid extraction	Please check the nucleic acid extraction procedure and re-extract the nucleic acid. If the original specimen is not available, a new specimen must be collected.

16. Symbols Used

The following symbols used on labels and packaging of this product conform to the harmonized standard EN980.

REF	Catalog Number
LOT	Lot/Batch Number
	Expiration Date
	Storage Conditions
	Manufactured by
CE IVD	Intended Use

17. Contact Information

Genmark Sağlık Ürünleri

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