

*Plex*Monkeypox

Product Code: 7118401 Reactions: 100 Storage and Stability:

Reagents are shipped on dry ice or ice packs. All kit components are stable at -25°C to -15°C; refer to expiry on the label. Excessive freeze/thawing is not recommended. Store protected from light at -25°C to -15°C.

Notes:

This product is for Research Use Only, not for use in diagnostic procedures.

RESEARCH USE ONLY



Store at -25°C to -15°C

Description

*Plex*Monkeypox is a 1 well, multiplex qPCR that simultaneously detects: the Orthopox L1R gene and the Monkeypox virus specific Serine Protease Inhibitor (SPI) gene. The mix is also combined with an endogenous control. The reagents are compatible with the following real-time detection systems: Roche LightCycler[®] 480 Instrument II (LC480 II), the Applied Biosystems[®] 7500 Fast (7500 Fast), Applied Biosystems[®] 7500 Fast Dx (7500 Fast Dx), Applied Biosystems[®] QuantStudio (QuantStudio), and the Bio-Rad CFX96[™] Dx (CFX96 Dx) and CFX96 Touch) Real-time PCR Detection Systems. The kit is for research use only (RUO) and is not intended for use in diagnostic procedures.

Components

Reagents	100 reactions	Cap colour
Monkeypox Mix, 20x	1 x 120 µl	Yellow
Plex Mastermix, 2x	1 x 1.2 ml	Blue
Nuclease Free Water	1 x 1 ml	Neutral

Store SpeeDx reagents separately from samples, i.e. template or nucleic acid handling room.

Recommended procedures:

Sample extraction

Samples should be extracted as total nucleic acid (TNA) prior to use with the kit.

Post extraction setup

1. qPCR Master mix setup 20.0 μl

Component	Supplied	Volume
Plex Mastermix, 2x	Yes	10.0 μL
Nuclease Free Water	Yes	4.0 μL
Monkeypox Mix, 20x	Yes	1.0 μL
Total volume (for 1 reaction)		15.0 μL

Recommended to vortex and centrifuge the components before making up the master mix.

Add 15.0 µl of the qPCR Master mix to each well.

Add 5.0 µl of sample to each well.

Programming and Results Interpretation

1. Roche LightCycler® 480 Instrument II (LC480 II)

Refer to LC480 II Instrument Operator's Manual

 Applied Biosystems® 7500 Fast (7500 Fast), Applied Biosystems® 7500 Fast Dx (7500 Fast Dx)

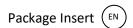
Refer to Applied Biosystems 7500 FAST/7500 FAST Dx manual

3. Applied Biosystems® QuantStudio (QuantStudio)

Refer to Quantstudio Real-Time PCR Instrument and Flex Real-Time PCR system software manual

Bio-Rad CFX96[™] Dx (CFX96 Dx) and CFX96 Touch[™] (CFX96 Touch)

Refer to CFX96 Dx and CFX96 Touch Real-Time PCR Detection Systems manual



2. Instrument Detection Formats

The channels used for LC480 II instrument are shown below.

Channel	Monkeypox Mix
465-510	Monkeypox
533-580	Endogenous Control
533-610	Orthopox

The channels used for 7500 Fast, and 7500 Fast Dx are shown below.

Channel	Monkeypox Mix
FAM	Monkeypox
JOE	Endogenous Control
Texas Red	Orthopox

The channels used for QuantStudio are shown below.

Channel	Monkeypox Mix
FAM	Monkeypox
JOE/VIC	Endogenous Control
Texas Red/ROX	Orthopox

The channels used for CFX96 Dx, and CFX Touch are shown below.

Channel	Monkeypox Mix
FAM	Monkeypox
HEX	Endogenous Control
Texas Red	Orthopox

3. Thermocycling Program

Create the following Cycling program

- Touch down cycling is for specific amplification of target
- Quantification cycling is for PCR amplification and fluorescence acquisition

Program Name	Cycles	Target °C	Hold
Polymerase activation	1	95°C	2 min
Touch down cycling: Step down	10	95°C	5 s
-0.5°C/Cycle		61°C − 56.5°C <mark>⁵</mark>	30 s
Quantification	40	95°C	5 s
cycling*: Acquisition/Detection		52°C+	40 s
Cooling	1	40°C	30 s

^⁵ Step size: -0.5°C/Cycle, Sec Target: 56°C

4. Results Interpretation

Perform results interpretation, as described in the instrument's operator manual.

For LC480 II SpeeDx Colour Compensation (CC) must be run and applied before analysis.

The **Plex**PCR[®] Colour Compensation kit (Cat no 90001, SpeeDx) can be provided upon request, please contact: sales @speedx.com.au

Target	Cq*	Result
Monkeypox	NEG	
Endogenous Control	POS/NEG	Other Orthopox virus detected
Orthopox	POS	•
Monkeypox	POS	
Endogenous Control	POS/NEG	Monkeypox virus detected
Orthopox	POS	
Monkeypox	NEG	Orthopox virus and Monkeypox virus
Endogenous Control	POS	not detected or Negative Control
Orthopox	NEG	reaction
Monkeypox	NEG	
Endogenous Control	NEG	No sample present, repeat test
Orthopox	NEG	
*Account off will pood to be determined by the upon		

^{*}Assay Cq cut-off will need to be determined by the user

⁺ Analysis mode: Quantification, Acquisition mode: Single