HELINI™

Ready to use

Human Immunodeficiency Virus- 1 RNA

Real-time PCR Primer Probe Mix

Instruction manual

info@helini.in
helinibiomolecules@gmail.com
www.helini.in

www.helini.in

Contents

1 vial of Primer Probe Mix [lyophilized]

1 vial of Positive control [50,000copies) lyophilized]

Storage & Expiry

Kits are stable for at least 12 months [-20C in the dark].

Dissolved reagents are stable for at least 6 months if stored protected from light and store at -20C.

Dissolved reagents can be stored long term at -20C [within expiry]. Avoid multiple freeze-thaw cycles.

Additional reagents required

For RNA virus, HELINI One step RT-PCR Probe Master Mix OR any reputed One step RT-PCR Probe Master Mix

HELINI Internal control template and Internal control Primer Probe Mix

HELINI Endogenous Primer Probe Mix [Human gene]

Sensitivity

This assay detects 10 genome equivalent copies or less per reaction. [Spiked plasmid control dilution]

Reagents Preparation

Primer Probe Mix

1. Spin down vials for 2 min at 10000rpm.
2. Add 140ul of Sterile distilled water or PCR grade water or Nuclease free water.
3. Incubate at room temperature for 5min.
4. Gently invert several times [15 – 20times] and spin down briefly.
5. It is ready to use now. Use 2.5ul per reaction for a 20ul or 25ul final qPCR reaction.

Positive control

1. Spin down vials for 2 min at 10000rpm.
2. Add 100ul of Sterile distilled water or PCR grade water or Nuclease free water.
3. Incubate at room temperature for 5min.
4. Gently invert several times [15 to 20times] and spin down briefly.
5. It is ready to use now. Use 5 to 10ul per reaction for a 20ul or 25ul final qPCR reaction.

RNA virus – Detection Mix

Components	20µl Final	25µl Final
One step RT-PCR Master Mix	8µl	8µl
RT-Taq enzyme mix	2µl	2µl
Pathogen/ Target Primer Probe Mix	2.5µl	2.5µl
Internal/Endogenous control* Primer Probe Mix [optional]	2.5µl	2.5µl
Purified RNA sample	5µl	10µl
Total reaction volume	20µl	25µl

* Internal control and Endogenous control primer probe mix are used to monitor the nucleic acid purification efficiency, biological status and PCR inhibition. Internal control is mostly recommended for all type of biological samples. However, endogenous control is recommended for the biological samples collected using swab. (Example, nasal, throat, Uro-genital swaps]

Negative Control setup
Add 10µl of PCR grade water or nuclease free water.

Qualitative Positive Control setup
Add 5 or 10µl of Positive control

Thermal Profile – RNA virus

	Step	Time	Temp
	Reverse transcriptase*	30min	42ºC
	Initial Denaturation / Taq enzyme activation*	15min	95ºC
45 cycles	Denaturation	20sec	95ºC
	Annealing/Data collection*	20sec	56ºC
	Extension	20sec	72ºC

* **Reverse transcriptase** & **Taq enzyme activation** duration may vary from company to company. Please read carefully and program as per their instructions.

Pathogen Detection Channel
FAM channel

Internal Control / Endogenous control Detection Channel
HEX/VIC/TET/Cy3/JOE

Reading the results

Test Sample FAM	Negative control FAM	Positive control FAM	Internal/ Endogenous Control HEX	Interpretation
Positive	Negative	Positive	**Positive**	**Pathogen DNA Detected**
Negative	Negative	Positive	**Positive**	**Pathogen DNA Not detected/beyond detection limit**
Negative	Negative	Negative	**Negative**	**Experiment fail**
Positive	Positive	Positive	**Positive**	**Experiment fail**

www.helini.in

In association with

Manufactured and Marketed by

HELINI *Biomolecules,*
Ohmlina, 26, 2nd Avenue,
Khuthubi Complex, Vettuvankeni,
Chennai 600115, Tamil Nadu.
www.helini.in
info@helini.in
info@helinibiomolecules.com
+91-44-24490433
+91-9382810333