

**HELINI™**



Ready to use

# Enterovirus

Real-time PCR Primer Probe Mix

**Instruction manual**

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## 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
<b>Purified RNA sample</b>	<b>5µl</b>	<b>10µl</b>
Total reaction volume	<b>20µl</b>	<b>25µl</b>

\* 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	42°C
	Initial Denaturation / Taq enzyme activation*	15min	95°C
	Denaturation	20sec	95°C
<b>45 cycles</b>	Annealing/Data collection*	20sec	56°C
	Extension	20sec	72°C

\* **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**

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

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