

HELINI™



Ready to use

Adenovirus [ADV]

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

HELINI Probe PCR Master Mix OR any reputed brand Probe PCR 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.

DNA virus - Detection Mix

| Components | 20µl Final | 25µl Final |
|--|------------|------------|
| Probe PCR Master Mix | 10µl | 10µl |
| Pathogen/ Target Primer Probe Mix | 2.5µl | 2.5µl |
| Internal/Endogenous control* Primer Probe Mix [optional] | 2.5µl | 2.5µl |
| Purified DNA 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 swabs)

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 – DNA virus

| | Step | Time | Temp |
|------------------|---|-------|------|
| 45 cycles | Initial Denaturation / Taq enzyme activation* | 15min | 95°C |
| | Denaturation | 20sec | 95°C |
| | Annealing/Data collection* | 20sec | 56°C |
| | Extension | 20sec | 72°C |

* **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

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 |

In association with

 **Gautham Pulavar Molecular Research**

Manufactured and Marketed by

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