

Quantiplus® Adenovirus Real-Time Quantitative PCR Kit



QT-ADV-25 : 25 rxns
 QT-ADV-50 : 50 rxns
 QT-ADV-100 : 100 rxns



PI/QTADV-02

Intended Use

Quantiplus® Adenovirus Real-Time Quantitative PCR Kit is a Real-Time PCR based in vitro diagnostic assay for quantitation of Adenovirus in human plasma, CSF, and urine. The kit contains Amplification Mix with specific Primers and Probes, Standards (ADQS1-ADQS4) and Internal Control. The kit contains a second amplification system to identify possible PCR inhibition by using an exogenous internal control (Huwel IC-A Mix) without affecting the analytical sensitivity of the assay.

Background Information

Human Adenoviruses (HAdVs) are ubiquitous double-stranded DNA viruses that cause a wide array of diseases in humans including pharyngitis, pneumonia, gastroenteritis, hemorrhagic cystitis, and keratoconjunctivitis. They also cause life-threatening opportunistic infections in immunocompromised individuals and are responsible for outbreaks in certain populations. Diagnosis of HAdV is mostly done by cell culture or antigen detection methods.

Kit Components

| Color Coding (Caps) | Contents | Description | 25 rxns (QT-ADV-25) | 50 rxns (QT-ADV-50) | 100 rxns (QT-ADV-100) |
|---------------------|-----------------------|---|---------------------|---------------------|-----------------------|
| Amber | Huwel Adeno Ready Mix | Probes and Primers for Adenovirus and Internal Control along with Amplification Mix | 1 x 375µL | 1 x 750µL | 2 x 750µL |
| Natural | Huwel IC-A Mix | Internal Control | 1 x 300 µL | 1 x 600 µL | 2 x 600 µL |
| Pink | Huwel ADQS1 | 2 X 10 ⁶ IU/µL | 1 x 100 µL | 1 x 100 µL | 2 x 100 µL |
| Pink | Huwel ADQS2 | 2 X 10 ⁵ IU/µL | 1 x 100 µL | 1 x 100 µL | 2 x 100 µL |
| Pink | Huwel ADQS3 | 2 X 10 ⁴ IU/µL | 1 x 100 µL | 1 x 100 µL | 2 x 100 µL |
| Pink | Huwel ADQS4 | 2 X 10 ³ IU/µL | 1 x 100 µL | 1 x 100 µL | 2 x 100 µL |
| White | Huwel PW | Purified Water | 1 x 500 µL | 1 x 500 µL | 1 x 1 mL |

Note: Please pay attention to the cap color coding and the tube contents.

Huwel PW: Molecular Biology Grade Purified Water.

Storage and Transportation Conditions

The kit should be transported at temperature below -20 °C. The kit is stable until the expiry date printed on the package, if the storage temperature is within -20 ±5 °C. More than 4X freezing and thawing cycles reduces the assay sensitivity. For intermittent usage the reagents should be frozen in aliquots.

Technical specifications

| | |
|-----------------------------|--|
| Target Sequence | Conserved region in <i>Hexon</i> gene of Adenovirus |
| Specificity | 100% |
| Sensitivity | 0.5 IU/µL (250 IU/mL or 250 copies/mL) |
| Linear Range | 2X10 ⁷ – 0.6 IU/µL (1 x 10 ¹⁰ – 3 x 10 ² IU/mL or 1 x 10 ¹⁰ – 3 x 10 ² copies/mL) |
| Reporting Units | IU/mL or Copies/ mL (1 IU = 1 copy) |
| Validated Specimen | Plasma |
| External Quality Assessment | QCMD EQA Panels |

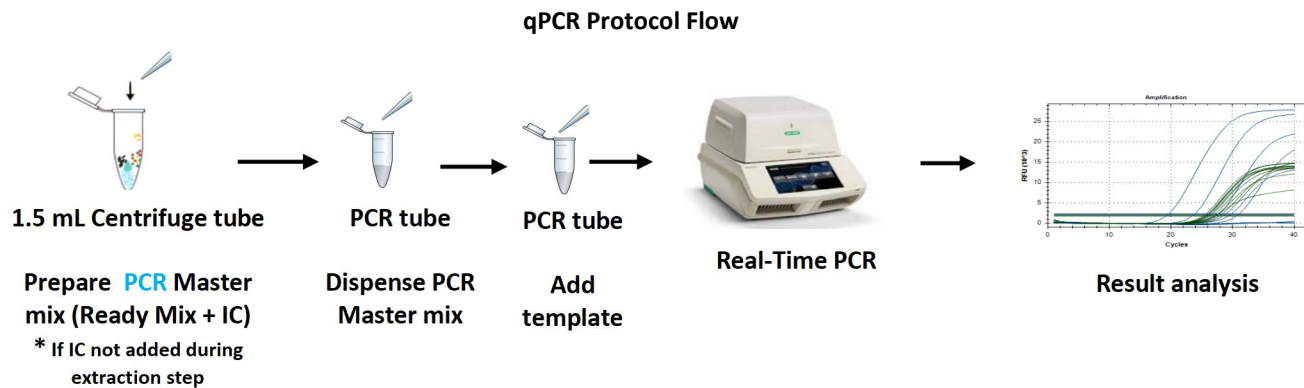
Assay Procedure

DNA Extraction

Quantiplus® Adenovirus Real-Time Quantitative PCR Kit has been validated using the following Viral DNA extraction kits:
Recommended sample volume for extraction and elution are as follows:

| S. No. | Name of the Extraction Kit | Recommended Sample volume for Extraction | Recommended Final Elution volume |
|--------|---|--|----------------------------------|
| 1. | Huwel Nucleic Acid Extraction Kit - Version 2.0 (Cat. No. HL-NAX-100) | 200 µL | 100 µL |
| 2. | QIAamp® DNA Blood Mini Kit (Cat. No. 51104) | 200 µL | 100 µL |

Note: Customer can also validate their own extraction process using other Viral DNA extraction Kits.
IC-A mix can be added at the extraction step or while setting up the PCR



Preparation of Reaction Master mix

| Components | Volume per reaction (for 26µL) |
|--|--------------------------------|
| Huwel Adeno Ready Mix | 15.0 |
| Huwel IC-A Mix (if not added at extraction step) | 1.0 |
| Extracted DNA/Huwel ADQS1- Huwel ADQS4/ Huwel PW | 10.0 |

It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. Close the tubes and centrifuge briefly before proceeding to the thermal cycler.

Cycling Conditions

| Steps | No. of cycles | Temperature (°C) | Time |
|--------------------------|---------------|------------------|---------|
| 1 (Initial denaturation) | 1 | 95 | 15 min. |
| 2 (PCR cycling) | 45 | 95 | 15 sec. |
| | | 60* | 1 min |

*Plate read/Data acquisition in **FAM** and **YAKIMA YELLOW/ VIC/ HEX** channels in Bio-Rad™, CFX 96 and Thermo Q55 Real-Time PCR System.

Sample analysis and Interpretation

The criteria for the acceptance of the assay should be met before the interpretation of the unknown sample results as described in Table 1 below and also the slope of the standard curve (standards in FAM channel) is between -3.1 to -3.6, (at least three standards should be included,) and PCR efficiency is between 90% to 110% (0.9 to 1.1). Interpret the results of unknown samples as mentioned in Table 2

Table 1:

| Control | FAM (ADV) | YAKIMA YELLOW/ VIC/ HEX (IC) |
|---|-----------|------------------------------|
| If Internal Control (IC-A Mix) is added during extraction | | |
| Standards (ADQS1 to ADQS4) | √ | - |
| Negative Control (NC) | - | - |
| If Internal Control (IC-A Mix) is added during preparation of reaction master mix | | |
| Standards (ADQS1 to ADQS4) | √ | √ |
| Negative Control (NC) | - | √ |

Table 2:

| S.No | FAM (ADV) | YAKIMA YELLOW/ VIC/HEX (IC) | Interpretation | Conclusion |
|------|-----------|-----------------------------|--|---|
| 1 | √ | √ | Adeno DNA detected within quantitation range | Proceed for further Analysis |
| 2 | √ | - | | |
| 3 | - | √ | | |
| 4 | - | - | Possible inhibition of PCR | Dilute the DNA sample (1:10) and repeat the Assay |

Viral load calculation (Conversion of IU/μL to IU/mL)

$$IU/mL = \frac{\text{Obtained IU}/\mu L \times \text{Elution Volume } (\mu L)}{\text{Sample volume in mL}}$$

For calculating the result of diluted sample (1:10); multiply the observed IU/mL value by dilution factor, 10

$$\text{Result of 1:10 diluted sample (IU/mL)} = \text{Dilution Factor (10)} \times \frac{\text{Result (IU}/\mu L) \times \text{Elution Volume } (\mu L)}{\text{Sample Volume (mL)}}$$

Reporting Comments

| Results in IU/mL | Comments |
|---------------------------|--|
| Target not detected | Adeno DNA not detected in the given sample |
| <300 | Adeno DNA detected but below the lower limit of the linear range of the assay. The reproducibility of the positive result is not assured |
| 300 to 1×10^{10} | Adeno DNA detected within the linear range of the assay |
| $>1 \times 10^{10}$ | Adeno DNA detected but above linear range of the assay, dilute the sample and repeat the assay for accurate result |

Validated Instruments

- Thermo QS5 Real-Time PCR System
- Bio-Rad™ CFX 96



HLSS Manufacturing Pvt Ltd
 Plot No's M14, M15, M16, TSIC Medical device park
 Sultanpur village, Ameenpur Mandal,
 SangareddyDist, TS-502319