Quantiplus® HIV-1 Quantitation Kit
(Real-time Quantitative PCR Kit)

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QT-HIV-25 : 25 rxns
QT-HIV-50 : 50 rxns
QT-HIV-100 :100 rxns

RUO

PI/QTHIV-02

Intended use

Quantiplus® HIV-1 Quantitation Kit is a Real-Time PCR based in vitro diagnostic assay for detection and quantitation of HIV genotypes (A, B, C, D, AE, F, G, AA - GH) in human plasma. The kit contains Amplification Mix with specific Primers and Probes, Standards (Huwel HIQS1-Huwel HIQS4) and Internal Control along with molecular biology grade water (to be used as a negative 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 immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes acquired immunodeficiency syndrome (AIDS), a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers. HIV infects vital cells in the human immune system such as helper T cells, macrophages, and dendritic cells. When CD4+ T cell numbers decline below a critical level, cell mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections. HIV differs from many viruses in that it has very high genetic variability. This diversity is a result of its fast replication cycle, with the generation of about 10^{10} virions every day, coupled with a high mutation rate. Detection of HIV-1 nucleic acid (RNA) by PCR can provide early evidence of HIV-1 infection (approximately 10-14 days after infection), when results of routine diagnostic assays are inconclusive. Clinical studies have indicated that detection of HIV-1 RNA in whole blood specimens by qPCR is highly sensitive (>95%) and specific (>98%) for the presence of early HIV-1 infection.

Kit components

Color Coding (Caps)	Contents	Description	25 rxns (QT-HIV-25)	50 rxns (QT-HIV-50)	100 rxns (QT-HIV-100)
Amber	Huwel HIV-1 Ready Mix	HIV-1, Internal Control Primers and Probes with amplification Mix	1 x 375 µL	1 x 750 µL	2 x 750 µL
Pink	RT Enzyme	cDNA synthesis reagent	1 x 25 µL	1 x 50 µL	1 x 100 µL
Natural	Huwel IC-A Mix	Internal Control	1 x 300 µL	1 x 600 µL	1 x 600 µL
Red	Huwel HIQS1	2 X 10^4 IU/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Red	Huwel HIQS2	2 X 10^3 IU/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Red	Huwel HIQS3	2 X 10^2 IU/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Red	Huwel HIQS4	2 X 10^1 IU/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Natural	qPCR Additive	PCR Reaction Enhancer	1 x 200 µL	1 x 200 µL	2 x 200 µL
White	Huwel PW	Purified water	1 x 500 µL	1 x 500 µL	2 x 500 µL

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

Huwel PW (Molecular biology grade nuclease free purified water)

Storage and Transportation Conditions

The kits should be transported at temperatures 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 reduce the assay sensitivity. For intermittent usage the reagents should be frozen in aliquots.

Technical Specification

Target Sequence	HIV *gag* gene
Specificity	100%
Sensitivity	80 IU/mL
Linear Range	0.3 IU/µL to 2 x 10^7 IU/µL
Reporting Units	1 IU/µL = 1.2 copies/µL
Validated Specimen	Plasma (K2EDTA-Blood)

Assay Procedure

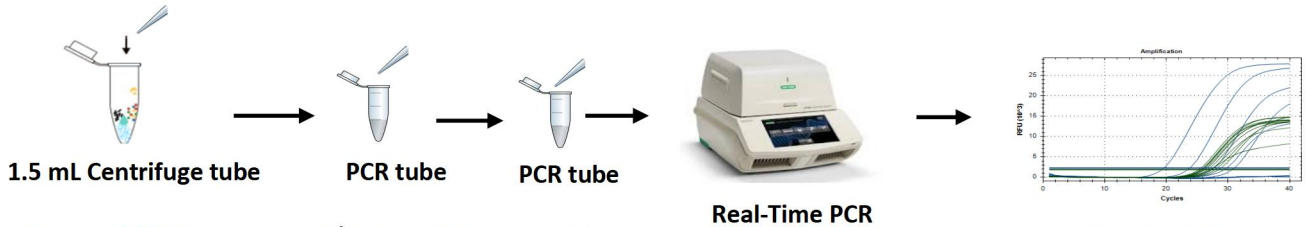
RNA Extraction

Quantiplus® HIV-1 Quantitation Kit (Real-Time Quantitative PCR Kit) has been validated using the following Viral RNA 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 Viral RNA Mini Kit (Cat. No. 52904)	140 µL	50 µL

Note: Customer can also validate their own extraction process using other Viral Nucleic acid extraction Kits.

qPCR Protocol Flow



Preparation of Reaction Master mix

Components	Volume per reaction (for 28 µL)
Huwel HIV-1 Ready Mix	15.0
RT Enzyme	1.0
qPCR Additive	1.0
Huwel IC-A Mix (If not added at extraction step)	1.0
Extracted RNA/ Huwel HIQS1-Huwel HIQS4 / 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 (cDNA Synthesis)	1	42	15 min.
2 (Initial denaturation)	1	95	15 min.
3 (PCR cycling)	5	94	10 sec.
		54	30 sec
		60	45 sec
4 (PCR cycling)	40	94	10 sec.
		56	30 sec
		60*	45 sec
*Plate read/Data acquisition in FAM and Yakima yellow/VIC/HEX channels			

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 ensure that 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 (HIV-1)	Yakima Yellow/VIC/HEX (Internal Control)
If Internal Control (IC-A Mix) is added during extraction		
Standards (HIQS1 to HIQS4)	√	-
Negative Control (NC)	-	-
If Internal Control (IC-A Mix) is added during preparation of reaction master mix		
Standards (HIQS1 to HIQS4)	√	√
Negative Control (NC)	-	√

Table 2:

S.No	FAM (HIV-1)	YAKIMA YELLOW/ HEX/ VIC (IC)	Fluorophore → / Interpretation ↓	Conclusion
1	√	√	HIV RNA detected within quantitation range	Proceed for further Analysis
2	√	-		
3	-	√	HIV RNA detected below quantitation limit	
4	-	-	Possible inhibition of PCR	Dilute the RNA sample (1:10) and repeat the Assay

Note: All the Target channels (FAM, and YAKIMA YELLOW/VIC/HEX) to be analyzed individually.

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

$$\text{IU/mL} = \frac{\text{Obtained IU/µL X Elution Volume (µL)}}{\text{Sample volume in mL}}$$

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

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

Reporting comments

Results in IU/mL	Comments
Target not detected	Sample does not contain HIV-1 RNA
<150	HIV-1 RNA detected but below the lower limit of the linear range of the assay. The reproducibility of the positive result is not assured
≥150 to 1X 10^{10}	HIV-1 RNA detected within the linear range of the assay
>1 X 10^{10}	HIV-1 RNA 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
- Rotor-Gene Q 5 plex

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