A Real-Time PCR (qPCR) Assays for Detection of Human Immunodeficiency Virus (HIV)

Product Insert

*ab*TES™ HIV qPCR I Kit
Kit Version: 1.0

RUO For research use only

REF 300241 (50 Reactions)
300242 (100 Reactions)

Store at -25°C to -15°C

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For use on Bio-Rad CFX96, Stratagene Mx3005P, and Qiagen RG Q.

1. Pathogen Information

HIV belongs to retrovirus family which causes the acquired immune deficiency syndrome (AIDS). Their main routes of transmission are unprotected sex, contaminated needles, breast milk and perinatal transmission. Upon transmission, HIV compromises the body's cell-mediated immunity and renders the body's susceptibility to opportunistic infections. Specifically, HIV attacks immune cells such as helper T-cells ($CD4^+$ T-cells), macrophages and dendritic cells and subsequently topples the body's defense system. Its lethality is reflected in the 2 million AIDS-related deaths in 2008 alone[1]. Although there is no definitive cure for the disease, treatment such as antiretroviral therapy has been quite promising especially is initiated early[2]. Hence, an accurate and rapid HIV test is essential for early diagnosis of HIV.

2. Test Description

The *ab*TES™ HIV qPCR I Kit is a real-time polymerase chain reaction (qPCR) kit for the detection of HIV. The kit contains all the necessary PCR reagents for rapid, sensitive and reproducible real-time detection of HIV using highly specific primer pairs and hydrolysis double-dye probes. The recommended sample types are serum and plasma (the use of heparinized plasma is not recommended). An **Internal Control (IC)** is also supplied to check for possible PCR inhibition.

3. Storage Conditions

The components of *ab*TES™ HIV qPCR I Kit should be stored in the dark, at -20°C in a **NON**-frost-free freezer. Frost-free freezers go through freeze-thaw cycles to remain frost-free and may cause accelerated degradation of enzymes and nucleic acids. Avoid repeated thawing and freezing (max 3 times) as this may lower the sensitivity. If reagents are used intermittently, it is suggested to keep the reagents frozen in aliquots.

4. Kit Components

Tubes	Items	300241 (50 rxns)	300242 (100 rxns)
1	2x RT-PCR Reaction Mix	1x 625 µL	2 x 625 µL
2	RT-Taq Enzyme Mix	1x 25 µL	2 x 25 µL
3	Primer/Probe Mix	1x 50 µL	2 x 50 µL
4	Internal Control	1x 200 µL	2 x 200 µL
5	Nuclease-free Water	1x 600 µL	2 x 600 µL
6	HIV Positive Control	1x 200 µL	1x 200 µL

5. Additional Materials Required but not Provided

- Disposable powder-free gloves
- Nucleic acid extraction kit
- Vortex mixer
- Pipettes and pipette tips with filter
- Desktop centrifuge with rotor
- Real-time thermal cycler
- 0.2 mL PCR tubes/ 96-well PCR plates
- Ice box/cooling block

6. Limitations and General Precautions

- The use of this product and its data interpretation are intended for personnel trained in real-time PCR techniques and *in vitro* diagnostics procedures only.
- It is advisable to analyze the real time PCR graph at the end of the run to determine the validity of the Ct data.
- Appropriate specimen collection, transport, storage and nucleic acid extraction procedures are required for reliable results.
- Wear disposable gloves, laboratory coats and eye protection when handling samples and reagents. Wash hands thoroughly thereafter.
- Use sterile pipette tips with filters and replace the tip for every procedure.
- Store and extract positive materials (specimens, controls and amplicons) separately from all other reagents and add to the reaction mix in a separate facility, if possible.
- Thaw all the components thoroughly at room temperature before starting an assay.
- When the components are thawed, mix the components and centrifuge briefly.
- Do not use the kit after its expiration date.

7. Procedures

7.1 Nucleic Acids (NA) Extraction

Standard NA extraction kits are compatible with this assay. Please carry out NA extraction as per instructed in the manufacturer's extraction kit manual.

7.2 PCR Reaction Setup

Thoroughly thaw all components, mix and spin briefly. Keep all components and samples on ice. Prepare your PCR reaction based on the following pipetting scheme:

Reagent	Volume per reaction			
	Test sample reaction	Positive control reaction	Negative control reaction	Non template reaction
2x RT-PCR Reaction Mix	12.5 µL	12.5 µL	12.5 µL	12.5 µL
RT/Taq Enzyme Mix	0.5 µL	0.5 µL	0.5 µL	0.5 µL
Primer/Probe Mix	1.0 µL	1.0 µL	1.0 µL	1.0 µL
Nuclease-Free Water	0.9 µL	0.9 µL	10.9 µL	11.0 µL
Internal Control	0.1 µL	0.1 µL	0.1 µL	-
Positive Control	-	10.0 µL	-	-
Extracted Test Sample	10.0 µL	-	-	-
Total Volume	**25µL**	**25 µL**	**25 µL**	**25 µL**

7.3 PCR Cycling Conditions

Gently mix the components and spin briefly. The following cycling conditions were established and validated on Bio-Rad CFX96, Stratagene Mx3005P and Qiagen Rotor-Gene Q. You may need to adjust these conditions for other real-time platforms. **FAM** (HIV) and **Texas Red** (IC) channels should be chosen and the fluorescence is measured at the annealing phase of each cycle.

Phase	Description	No. of Cycles	Temperature	Duration
1	cDNA synthesis	1	55 °C	30 min
2	Taq activation	1	95 °C	2 min 30s
3	Amplification	42	95 °C	17 sec
			*65 °C	31 sec
			68 °C	32 sec

**Data acquisition at annealing phase*

8. Interpretation of Data

A sample will be considered as having a positive result if the fluorescence level is higher than the threshold value and will be considered negative, otherwise.

As the kit includes an internal control, all samples that are negative for HIV (FAM) should be positive at the internal control channel (Texas Red). A negative internal control in this case may indicate a presence of PCR inhibitors in the sample or a problem with the PCR reaction. The internal control may not necessarily be positive if the sample is positive for HIV due to competition of reagents.

Result	HIV Ct (FAM)	Internal Control (Texas Red)
Negative	-	+
Positive	+	+ or -
Indeterminate	-	-

9. Troubleshooting

9.1 No signal observed with positive control

- Check programmed temperature settings against the protocol given.
- Affirm if proper storage was done and check the expiry date on the kit; use a newer kit to repeat experiments if needed.
- PCR inhibition has possibly occurred: re-purify RNA sample to remove inhibitors and repeat PCR, if needed.

9.2 Signal detected for negative control

- A contamination in the reagents or samples is highly possible.
- Repeat experiment protocol and take steps to locate source of contamination.

9.3 Weak or no signal of the internal control and no sign detection in analytical channel as well

- A possible PCR inhibition has occurred. Re-extract the sample to remove inhibitors and repeat PCR, if needed.
- Affirm if proper storage was done and check the expiry date on the kit. Use a new kit to repeat the test if needed.

10. Explanation of Symbols

RUO	For research use only
REF	Catalogue number
15°C / -25°C	Store at -25°C to -15°C
	Manufacturer
LOT	Lot number
	Use by
Σ	Contains sufficient for <n> tests

11. References

1. World Health Organization. 2009. AIDS epidemic update : November 2009. UNAIDS/09.36E/JC1700E. World Health Organization, Geneva, Switzerland.

2. Severe P, Juste MA, Ambroise A, Eliacin L, Marchand C, Apollon S, Edwards A, Bang H, Nicotera J, Godfrey C, Gulick RM, Johnson WD Jr, Pape JW, Fitzgerald DW. Early versus standard antiretroviral therapy for HIV-infected adults in Haiti. N Engl J Med. 2010 Jul 15;363(3):257-65.

Electronic copy of the product insert can be downloaded at http://aitbiotech.com/hiv/

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