A Real-Time PCR (qPCR) Assay for the Detection and Differentiation of HSV-1, HSV-2 and VZV

Product Insert

*ab*TES™ HSV/VZV qPCR I Kit
Kit Version: 2.1

RUO For research use only

REF 300425 (50 Reactions)
300426 (100 Reactions)

Store at -25°C to -15°C

AITbiotech Pte Ltd
25 Pandan Crescent, #05-15
TIC TECH Centre,
Singapore 128477

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

1. Pathogen Information

The Herpesviridae are a large family of DNA viruses that cause diseases in animals, including humans. The Herpesviridae family is subdivided into 3 subfamilies. The Alphaherpesvirinae, Betaherpesvirinae and Gammaherpesvirinae. The Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2) and Varicella Zoster Virus (VZV) belongs to the Alphaherpesvirinae subfamily, which is distinguished by reproducing more quickly than other subfamilies of Herpesviridae.

Infection with the Herpes Simplex Virus is categorized based on the site of infection, with oral herpes being the most common and genital herpes the second most common. HSV-1 is the main cause of oral herpes and HSV-2 the main cause of genital herpes. Varicella Zoster Virus is the cause of chicken-pox and shingles.

2. Test Description

The *ab*TES™ HSV/VZV qPCR I Kit is a real-time PCR (qPCR) kit for the detection and differentiation of HSV-1, HSV-2 and VZV. This kit contains all the necessary PCR reagents for rapid, sensitive and reproducible real-time detection and differentiation of the three members of the Herpes family using highly specific primer pairs and double-dye hydrolysis probes.

3. Storage Conditions

The components of *ab*TES™ HSV/VZV 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	300425 (50 rxns)	300426 (100 rxns)
1	Enzyme/Reaction Mix	1x 300 µL	2 x 300 µL
2	Primer/Probe Mix	1x 100 µL	2 x 100 µL
3	Internal Control	1x 500 µL	2 x 500 µL
4	Nuclease-free Water	1x 600 µL	2 x 600 µL
5	HSV/VZV PC ALL	1x 100 µL	1x 100 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
Enzyme/Reaction Mix	6 µL	6 µL	6 µL	6 µL
Primer/Probe Mix	2 µL	2 µL	2 µL	2 µL
Nuclease-Free Water	10 µL	10 µL	15 µL	17 µL
Internal Control	2 µL	2 µL	2 µL	-
Positive Control	-	5 µL	-	-
Extracted Test Sample	5 µL	-	-	-
Total Volume	**25 µL**	**25 µL**	**25 µL**	**25 µL**

7.3 PCR Cycling Conditions

The following cycling conditions were established and validated on Bio-Rad CFX96. You may need to adjust these conditions for other real-time platforms. **FAM** (HSV-1), **ROX** (HSV-2), **Cy5** (VZV) and **HEX** (IC) channels should be chosen and the fluorescence is measured at the end of annealing-extension phase of each cycle.

Phase	Description	No. of Cycles	Temperature	Duration
1	*Taq* activation	1	95 °C	5 min
2	Amplification	45	95 °C	10 sec
			*60 °C	20 sec

**Data acquisition at annealing phase*

8. Interpretation of Data

A sample is considered as positive if the fluorescence level is higher than the threshold value.

The result is regarded as true negative for HSV-1, HSV-2 and VZV when the FAM, ROX and Cy5 channels are negative and the HEX Internal Control channel is positive.

Result	HSV-1 (FAM)	HSV-2 (ROX)	VZV (Cy5)	IC (HEX)
HSV-1 positive	+	-	-	+ / -
HSV-2 positive	-	+	-	+ / -
VZV positive	-	-	+	+ / -
HSV-1, HSV-2 & VZV negative	-	-	-	+
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; Repeat the run with a new kit if needed.
- PCR inhibition has possibly occurred: re-purify DNA 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. Repeat the run with a new kit if needed.

10. Explanation of Symbols

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

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

AITbiotech Pte Ltd
25 Pandan Crescent #05-15, TIC TECH Centre, Singapore 128477
(65) 6778 6822 • sales@aitbiotech.com • www.aitbiotech.com