A Multiplex Real-Time PCR (qPCR) Assay for Detection of Four Dengue (DENV) Serotypes Viruses

Product Insert

*ab*TES™ DEN 4 qPCR I Kit
Kit Version: 2.2

CE IVD

REF 300155 (50 Reactions)
300156 (100 Reactions)

Store at -25°C to -15°C

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For use on Bio-Rad CFX96 and *ab*CyclerQ only.

1. Pathogen Information

Dengue fever and Dengue hemorrhagic fever (DHF) are viral diseases transmitted by *Aedes* mosquitoes, usually *Aedes aegypti*. They are found in most tropical and subtropical areas of the world, and have become the most common arboviral disease of humans. Human infections are acquired by the bite of infected *Aedes aegypti* mosquitoes, and epidemics are sustained by human-mosquito-human transmission. There are four virus serotypes, Dengue 1 (DENV-1), Dengue 2 (DENV-2), Dengue 3 (DENV-3) and Dengue 4 (DENV-4), each serotype is sufficiently different so that there is no cross-protection and epidemics caused by multiple serotypes can occur.

2. Test Description

The *ab*TES™ DEN 4 qPCR I Kit is a multiplex real-time polymerase chain reaction (qPCR) kit for the simultaneous detection of DENV-1, DENV-2, DENV-3 and DENV-4 in one reaction tube. The kit contains all the necessary PCR reagents for rapid, sensitive and reproducible real-time detection of four serotypes of Dengue virus RNA (DENV-1, DENV-2, DENV-3, and DENV-4) using highly specific primer pairs and double-dye hydrolysis probes. The kit adopts one tube system and the recommended human sample types are serum and plasma. An **Internal control (IC)** is also supplied to check for possible PCR inhibition.

3. Storage Conditions

The components of *ab*TES™ DEN 4 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 will be used intermittently, it is suggested to keep the reagents frozen in aliquots.

4. Kit Components

Tubes	Items	300155 (50 rxns)	300156 (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 75 µL	2 x 75 µL
4	Internal Control	1x 200 µL	2 x 200 µL
5	Nuclease-free Water	1x 600 µL	2 x 600 µL
6	DEN ALL-PC	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 or mix components from different lots.

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 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	1.5 µL	1.5 µL	1.5 µL	1.5 µL
Nuclease-Free Water	4.5 µL	4.5 µL	4.5 µL	10.5 µL
Internal control	1.0 µL	1.0 µL	1.0 µL	-
Positive Control	-	5.0 µL	-	-
Extracted Test Sample	5.0 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 and *ab*CyclerQ. You may need to adjust these conditions for other real-time platforms. **Cy5** (DENV-1), **FAM** (DENV-2), **Texas Red** (DENV-3), **Quasar 705/Alexa Fluor 680** (DENV-4) and **HEX** (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	53 °C	10 min
2	*Taq* activation	1	95 °C	2 min 30 sec
3	Amplification	42	95 °C	17 sec
			*59 °C	31 sec
			68 °C	32 sec

**Data acquisition at annealing phase*

8. Performance Characteristics

All reactions for performance characteristics were performed using the *ab*TES™ DEN/CHIKU 5 qPCR I Kit on the Bio-Rad CFX96 and *ab*CyclerQ. The reaction composition, cycling condition and primer/probe design for the DENV-1, DENV-2, DENV-3 and DENV-4 targets of the *ab*TES™ DEN/CHIKU 5 qPCR I Kit are identical to this kit. The data generated is therefore considered a good representative of the performance of this kit.

8.1. Analytical Sensitivity

Analytical sensitivity (limit of detection) is defined as the lowest concentration at which the assay can detect with a positivity rate of at least 95%.

The analytical sensitivity of the assay was determined by analyzing a dilution series of Commercial RNA Controls (Vircell) from 0.5 to 500 copies/µl. The testing was carried out in either 10-replicates (for concentrations ≥50 copies/µl) or 14-replicates (for concentrations <50 copies/µl). The analytical sensitivity was estimated by probit analysis using SPSS release 16.0.0.

The analytical sensitivity (not in consideration of extraction) was determined to be the following:

Target	Detecing channel	Analytical Sensitivity (95% confidence)	
		CFX96	*ab*CyclerQ
DENV-1	Cy5	10.30 copies/µL	0.55 copies/µl
DENV-2	FAM	2.82 copies/µL	12.5 copies/µl
DENV-3	Texas Red	23.77copies/µL	14.80 copies/µl
DENV-4	Quasar705 /Alexa Fluor 680	3.22 copies/µL	0.73 copies/µl

8.2. Analytical Specificity

The assay was tested for potential cross-reactivity against the following panel of 20 organisms. No cross-reactivity was observed.

Organisms Tested for Analytical Specificity	
Chlamydophila pneumoniae (AR-39)	Paravovirus b19
Cytomegalovirus (AD-169)	*Staphylococcus aureus* (FPR3757)
Enterovirus 71 (BrCr)	*Streptococcus pneumoniae*
Epstein-barr virus (HTB-62)	Varicella zoster virus (Ellen)
Hepatitis B virus	West nile virus (New York 99)
HIV-1	Dengue 1 virus
Human herpes virus 6	Dengue 2 virus
Influenza A virus (pdm H1N1-2009)	Dengue 3 virus
Influenza B virus	Dengue 4 virus
Mycobacterium tuberculosis (H37Rv)	Chikungunya virus

8.3. Precision/Reproducibility

The inter-assay (variability between different runs) and intra-assay (variability within one run) precision was determined by performing the assay once per day in 5-replicates over a period of 2 days for 5 samples of different concentrations (total of 50 reactions per target).

For all the 4 targets, the qualitative results of all 50 reactions were 100% reproducible.

The coefficient of variation (CV) of the cycle threshold (Ct) for the intra- and inter-assay precision is as follows:

Inter-assay precision data showing %CV (calculated from Ct values) at each concentration:

	2000 copies/µl	1000 copies/µl	500 copies/µl	100 copies/µl	50 copies/µl
DENV-1	1.7%	1.7%	1.0%	1.2%	1.2%
DENV-2	0.5%	0.7%	0.7%	0.7%	0.8%
DENV-3	1.1%	0.8%	1.2%	1.0%	1.1%
DENV-4	1.3%	1.0%	0.5%	1.0%	1.2%

Intra-assay precision data showing %CV (calculated from Ct values) at each concentration:

	2000 copies/µl	1000 copies/µl	500 copies/µl	100 copies/µl	50 copies/µl
DENV-1	1.5%	1.8%	1.0%	1.2%	1.2%
DENV-2	0.5%	0.6%	0.8%	0.5%	0.7%
DENV-3	0.7%	0.7%	0.9%	1.1%	1.0%
DENV-4	1.3%	0.9%	0.5%	1.0%	1.3%

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8.4. Diagnostic Evaluation

The assay was evaluated using 177 clinical samples previously serotyped by nested PCR and or Sanger sequencing and or third real-time PCR assay.

Diagnostic sensitivity and specificity of **DENV-1**:

Reference Method	*ab*TES (*n*=177)		Sensitivity/ Specificity
	DENV-1 Positive	DENV-1 Negative	
DENV-1 Positive	81	0	100% sensitivity
DENV-1 Negative	0	96	100% specificity
Total	81	96	

Diagnostic sensitivity and specificity of **DENV-2**:

Reference Method	*ab*TES (*n*=177)		Sensitivity/ Specificity
	DENV-2 Positive	DENV-2 Negative	
DENV-2 Positive	39	2	95.1% sensitivity
DENV-2 Negative	3	133	97.8% specificity
Total	42	135	

Diagnostic sensitivity and specificity of **DENV-3**:

Reference Method	*ab*TES (*n*=177)		Sensitivity/ Specificity
	DENV-3 Positive	DENV-3 Negative	
DENV-3 Positive	31	2	93.9% sensitivity
DENV-3 Negative	5	139	96.5% specificity
Total	36	141	

Diagnostic sensitivity and specificity of **DENV-4**:

Reference Method	*ab*TES (*n*=177)		Sensitivity/ Specificity
	DENV-4 Positive	DENV-4 Negative	
DENV-4 Positive	12	1	92.3% sensitivity
DENV-4 Negative	0	164	100% specificity
Total	12	165	

9. Interpretation of Data

A sample will be considered as having a positive result if an amplification signal is detected in the respective fluorescence channel.

The result is regarded as true negative for DENV-1, DENV-2, DENV-3 and DENV-4 when the CY5, FAM, Texas Red and Q705 are negative and the HEX IC channel is positive.

Target	DENV-1 (Cy5)	DENV-2 (FAM)	DENV-3 (Texas Red)	DENV-4 (Q705)	IC (HEX)
DENV-1 positive	+	-	-	-	-/+
DENV-2 positive	-	+	-	-	-/+
DENV-3 positive	-	-	+	-	-/+
DENV-4 positive	-	-	-	+	-/+
DEN ALL Positive-Control	+	+	+	+	+
Non-template Control	-	-	-	-	+

10. Troubleshooting

10.1. No signal observed with positive control in analytical channel and internal control channel

- 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.
- Check proper volume of the reagent added during the PCR setup.

10.2. Signal detected for negative control

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

10.3. No signal observed with samples in analytical channel and internal control channel

- PCR inhibition has possibly occurred: Re-purify samples to remove inhibitors and repeat PCR, if needed.

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11. Explanation of Symbols

IVD	*In vitro* diagnostic medical device
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/denv-chikv-zikv/

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