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

Product Insert

*ab*TES™ DEN 4 PRO qPCR I Kit
Kit Version: 2.0

CE IVD

REF 300185 (50 Reactions)
300186 (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 PRO 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. The *ab*TES™ DEN 4 PRO qPCR I Kit enables detection of mRNA of the human housekeeping gene, GAPDH as an **Internal Control (IC)** to identify possible PCR inhibitions and sampling problems.

3. Storage Conditions

The components of *ab*TES™ DEN 4 PRO 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	300185 (50 rxns)	300186 (100 rxns)
1	2x RT-PCR Reaction Mix	1x 500 µL	2 x 500 µL
2	RT/*Taq* Enzyme Mix	1x 20 µL	2 x 20 µL
3	Primer/Probe Mix	1x 100 µL	2 x 100 µL
4	Nuclease-free Water	1x 600 µL	2 x 600 µL
5	DEN PRO 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
- Icebox/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 tips 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	Non template control reaction
2x RT-PCR Mix	10.0 µL	10.0 µL	10.0 µL
RT/ *Taq* Enzyme Mix	0.4 µL	0.4 µL	0.4 µL
Primer/Probe Mix	2.0 µL	2.0 µL	2.0 µL
Nuclease-Free Water	2.6 µL	2.6 µL	7.6 µL
Positive Control	-	5 µL	-
Extracted Test Sample	5 µL	-	-
Total Volume	**20 µL**	**20 µL**	**20 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. **FAM** (DENV-1), **ROX** (DENV-2), **HEX** (DENV-3), **Cy5** (DENV-4) and **Quasar 705/Alexa Fluor 680** (GAPDH) 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	52 °C	15 min
2	Initial Denaturation	1	95 °C	2 min 30secs
3	Amplification	45	95 °C	15 secs
			*60 °C	30 secs

**Data acquisition at annealing phase*

8. Performance Characteristics

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 *in vitro transcribe (*IVT*)* RNA from 0.004 to 2000 copies/µl for DENV-1, 0.00004 copies/µl to 2000 copies/µl for DENV-2, 0.0001 copies/µl to 2000 copies/µl for DENV-3 and 0.004 copies/µl to 2000 copies/µl for DENV-4. The testing was carried out in either 10-replicates (for concentrations ≥200 copies/µl) or 14-replicates (for concentrations ≤20 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	Detection Channel	Analytical Sensitivity (95% confidence)
DENV-1	FAM	0.48 copies/µL
DENV-2	ROX	0.54 copies/µL
DENV-3	HEX	0.58 copies/µL
DENV-4	Cy5	0.52 copies/µL

8.2. Analytical Specificity

The assay was tested for potential cross-reactivity against the following panel of 10 organisms. No cross-reactivity was observed.

Organisms Tested for Analytical Specificity	
Dengue Virus 1	Yellow Fever Virus
Dengue Virus 2	St Louis Encephalitis Virus
Dengue Virus 3	West Nile Virus
Dengue Virus 4	Parvovirus B19
Chikungunya Virus	Zika Virus

9. 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 4 samples of different concentrations (total of 40 reactions per target).

For all the 4 targets, the qualitative results of all 40 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	200 copies/µl	20 copies/µl	2 copies/µl
DENV-1	0.81%	0.19%	0.64%	0.77%
DENV-2	0.27%	0.27%	0.51%	1.85%
DENV-3	0.51%	0.19%	0.74%	0.67%
DENV-4	0.25%	0.39%	0.27%	1.16%

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

	2000 copies/µl	200 copies/µl	20 copies/µl	2 copies/µl
DENV-1	0.83%	0.17%	0.55%	0.80%
DENV-2	0.26%	0.18%	0.47%	1.73%
DENV-3	0.46%	0.20%	0.74%	0.62%
DENV-4	0.18%	0.32%	0.24%	1.02%

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9.1. Diagnostic Evaluation

A retrospective study was performed using the *ab*TES™ DEN 4 PRO qPCR I Kit for clinical diagnostic evaluation. A total of 25 clinically extracted RNA samples were tested and the outcome was matched for concordance with previously typed result. The clinical diagnostic specificity and sensitivity was summarized in the table below:

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

Reference Method	*ab*TES (*n*=25)		Sensitivity/ Specificity
	DENV-1 Positive	DENV-1 Negative	
DENV-1 Positive	8	0	100% sensitivity
DENV-1 Negative	0	17	100% specificity
Total	8	17	

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

Reference Method	*ab*TES (*n*=25)		Sensitivity/ Specificity
	DENV-2 Positive	DENV-2 Negative	
DENV-2 Positive	9	0	100% sensitivity
DENV-2 Negative	0	16	100% specificity
Total	9	16	

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

Reference Method	*ab*TES (*n*=25)		Sensitivity/ Specificity
	DENV-3 Positive	DENV-3 Negative	
DENV-3 Positive	5	0	100% sensitivity
DENV-3 Negative	0	20	100% specificity
Total	5	20	

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

Reference Method	*ab*TES (*n*=25)		Sensitivity/ Specificity
	DENV-4 Positive	DENV-4 Negative	
DENV-4 Positive	4	0	100% sensitivity
DENV-4 Negative	0	21	100% specificity
Total	4	21	

10. 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 FAM, ROX, HEX and Cy5 are negative, and the Q705 (GAPDH) channel is positive.

Target	DENV-1 (FAM)	DENV-2 (ROX)	DENV-3 (HEX)	DENV-4 (Cy5)	GAPDH (Q705)
DENV-1 positive	+	-	-	-	-/+
DENV-2 positive	-	+	-	-	-/+
DENV-3 positive	-	-	+	-	-/+
DENV-4 positive	-	-	-	+	-/+
DENGUE ALL PC	+	+	+	+	-
Indeterminate	-	-	-	-	-

11. Troubleshooting

11.1. No signal observed with positive control in the 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 the proper volume of the reagent added during the PCR setup.

11.2. Signal detected for negative control

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

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

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

12. Explanation of Symbols

IVD	*In vitro* diagnostic medical device
REF	Catalogue number
	Store at -25°C to -15°C
	Manufacturer
EC REP	Authorized representative in the European community
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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