

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

Product Insert
abTES™ DEN 4 qPCR II Kit
Kit Version: 2.2



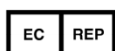
REF 300157 (50 Reactions)
300158 (100 Reactions)



Store at -25°C to -15°C



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



SIA “Medevice Group”
Jurmālas gatve 32, Rīga,
LV-1083, Latvia.
www.medevice-group.com



For use on ABI 7500/ 7500 Fast.

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 abTES™ DEN 4 qPCR II 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 abTES™ DEN 4 qPCR II 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	300157 (50 rxns)	300158 (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

Gently mix the components and spin briefly. The following cycling conditions were established and validated on the ABI 7500/ 7500 Fast. You may need to adjust these conditions for other real-time platforms. **Cy5** (DENV-1), **FAM** (DENV-2), **Texas Red** (DENV-3), **TAMRA** (DENV-4) and **VIC** (IC) channels should be chosen, and the fluorescence is measured at the annealing phase of each cycle.

ABI 7500/ 7500 Fast Settings	
Ramp speed	Standard
Passive reference	None

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 abTES™ DEN/CHIKU 5 qPCR I Kit on the Bio-Rad CFX96. The reaction composition, cycling condition and primer/probe design for the DENV-1, DENV-2, DENV-3 and DENV-4 targets of the abTES™ 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 (Viracell) 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	Detection Channel	Analytical Sensitivity (95% confidence)
DENV-1	Cy5	10.30 copies/µL
DENV-2	FAM	2.82 copies/µL
DENV-3	Texas Red	23.77 copies/µL
DENV-4	TAMRA	3.22 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	
<i>Chlamydomonas pneumoniae</i> (AR-39)	Parvovirus b19
Cytomegalovirus (AD-169)	<i>Staphylococcus aureus</i> (FPR3757)
Enterovirus 71 (BrCr)	<i>Streptococcus pneumoniae</i>
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
<i>Mycobacterium tuberculosis</i> (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%

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	abTES (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	abTES (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	abTES (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	abTES (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, TAMRA channel are negative and the VIC channel is positive for internal control.

Target	DENV-1 (Cy5)	DENV-2 (FAM)	DENV-3 (Texas Red)	DENV-4 (TAMRA)	IC (VIC)
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.

11. Explanation of Symbols

	In vitro diagnostic medical device
	Catalogue number
	Store at -25°C to -15°C
	Manufacturer
	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/>