A Multiplex Real-Time PCR (qPCR) Assay for Detection of Chikungunya (CHIKU) Virus

Product Insert

*ab*TES™ CHIKU qPCR I Kit
Kit Version: 2.1

RUO For research use only

REF 300161 (50 Reactions)
300162 (100 Reactions)

Store at -25°C to -15°C

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For use on Bio-Rad CFX96 and Stratagene Mx3005P.

1. Pathogen Information

While Dengue is the commonest and the most rapidly spreading mosquito-borne viral disease in the world, Chikungunya (CHIKU) fever has recently re-emerged after an interval of several decades to affect millions of people, particularly in India and Southeast Asia. CHIKU epidemic cycle is similar to that of Dengue, characterized by sudden onset of chills, fever, headache, nausea, vomiting and rash.

2. Test Description

The *ab*TES™ CHIKU qPCR I Kit is a multiplex real-time polymerase chain reaction (qPCR) kit for the detection of CHIKU. The kit contains all the necessary PCR reagents for rapid, sensitive and reproducible real-time detection of Chikungunya virus RNA 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.

3. Storage Conditions

The components of *ab*TES™ CHIKU 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	300161 (50 rxns)	300162 (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	Positive Control	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
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	5.4 µL	5.4 µL	5.4 µL	10.5 µL
Internal control	0.1 µL	0.1 µL	0.1 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 Bio-Rad CFX96 and Stratagene Mx3005P. You may need to adjust these conditions for other real-time platforms. **HEX** (CHIKU) 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	53 °C	10 min
2	*Taq* Activation	1	95 °C	2 min 30 sec
3	Amplification	42	95 °C	20 sec
			*59 °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.

9. Troubleshooting

9.1 No signal observed with positive control and negative 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.
- Check proper volume of the reagent added during the PCR setup.

9.2 Signal detected for negative control

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

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. Performance Characteristics

10.1. 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 quantitated virus RNA from 0.000625 PFU/µL to 50 PFU/µL. The testing were carried out in either 10-replicates (for concentrations ≥0.5 PFU/µl) or 14-replicates (for concentrations <0.5 PFU/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	CFX96 Channel	Analytical Sensitivity (95% confidence)
CHIKU	HEX	0.0043 PFU/µL

10.2. 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

10.2. 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).

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 are as follows:

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

	500 PFU/µl	50 PFU/µl	5 PFU/µl	0.5 PFU/µl	0.05 PFU/µl
CHIKV	0.9%	1.3%	0.9%	0.9%	1.2%

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

	500 PFU/µl	50 PFU/µl	5 PFU/µl	0.5 PFU/µl	0.05 PFU/µl
CHIKV	0.8%	1.1%	0.7%	0.8%	1.1%

10.3. Diagnostic Evaluation

The assay was evaluated using 16 serum samples previously serotyped by nested-PCR and/or a second real-time RT-PCR assay.

Diagnostic sensitivity and specificity of **CHIKU**:

Reference Method	*ab*TES (*n*=16)		Sensitivity/ Specificity
	CHIKU Positive	CHIKU Negative	
CHIKU Positive	4	0	100% sensitivity
CHIKU Negative	0	12	100% specificity
Total	4	12	

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11. 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/denv-chikv-zikv/

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