CE IVD

DiAGSure DIAGNOSTIC
for the people, by the people

DiAGSure® Dengue TaqMan PCR Kit

Catalogue No.: GDQ6080- 96R
Pack size: 96 Tests

Kit Components:

Color Coding (Caps)	Reagents	Description	Volume	No. of vials
Red	UNG+ OS Master Mix	Enzyme mix	50 µL	8
Amber	Primer-Probe Mix	Amplification Reagent for Dengue virus	25 µL	8
Yellow	Positive Control (PC)	Positive Control	60 µL	2
Blue	Negative Control (NC)	Negative Control	1 mL	1
Green	Internal Control (IC)	Heterologous Internal control	500 µL	2
White	NFW	Nuclease-free water	1 mL	1

Intended Use:
The DiAGSure® Dengue TaqMan PCR Kit is an in vitro diagnostic (IVD) real-time reverse transcriptase polymerase chain reaction (qRT-PCR) test involving TaqMan chemistry intended for the qualitative detection of Dengue virus RNA belonging to any of the four genotypes (DenV1-4) in human serum or plasma. Highly conserved regions in the viral capsid gene (cPrM) and a polyprotein (POLY) gene have been targeted in the same channel to target all four DenV genotypes. This two-plex PCR includes a heterologous internal control (IC) in a single- tube reaction.

Estimated operating time:
~1 hr 15 mins

Kit Stability:
- Kit is shipped on dry ice and should be stored immediately upon receipt at –20°C in a constant temperature freezer. Please refer to product label for final expiry date.
- Repeated thawing and freezing (>7x) should be avoided, as this may reduce sensitivity.
- If the reagents are to be used only intermittently, they should be frozen in aliquots.

- Kit stability: 18 months from date of manufacturing

Specimen handling & Storage:

After sample collection, perform the test on the same day. Otherwise, store it in the following condition: 2 to 8˚C for no more than 24 hours Store below -20˚C for no more than 3 days. Samples can be stored for a long time below -70 ˚C. Repeated freezing-thawing of samples should be avoided.

Transportation: The foam box is sealed with ice for transportation.

Operation procedure:

A. Sample solution preparation:

Clinical samples are extracted according to the corresponding requirements and steps with the viral RNA extraction kit, and the extracted RNA could be directly used for detection. During RNA isolation, add 5 µL of the Internal Control (IC) to the lysis buffer and proceed normally with the extraction steps. If samples are not immediately tested after extraction, they can also be stored at -70°C, and repeated freezing and thawing should be avoided. It is advised to start amplification with at least 100 ng RNA. Note: Freshly extracted RNA should be used. Otherwise, RNA should be stored in alcohol at -80°C. Since RNA is a labile biomolecule, improper handling and storage can lead to significant loss of titer and may affect the kit's sensitivity.

B. Preparation of amplification reagent:

Take the reagents out from the -20˚C freezer and thaw them on ice. After thawing, briefly vortex the reagents followed by a short spin. The PCR reagents should be kept on ice.

C. TaqMan qPCR protocol:

Per sample, set up a single reaction according to the following table:

Components	Volume per reaction
UNG+ OS Master Mix	4 µL
Primer-Probe Mix	2 µL
Positive Control (PC)/ Sample RNA/ Negative Control (NC)	10 µL
NFW	4 µL
Total Volume	20 µL

*In case the Internal Control (IC) is not added during RNA isolation, it can be directly added to the master mix at 0.5 µL per reaction without any significant change in reaction volume.

D. Real-time PCR Instrument set up:

Set up the PCR tubes/strips/plate in the real time machine and label the sample slots accordingly. Select the sample type (Unknown/PC/NTC) and select the acquisition channel for each slot as follows:

Target	Channel	Source wavelength (nm)	Detection wavelength (nm)
Dengue virus	FAM (Green)	470	510
Reference gene (IC)	VIC (Yellow)	538	554

E. PCR cycling conditions:

Step	Temperature (°C)	Time	No. of cycles
RT reaction	50	8 min	1
Hold stage	95	3 min	1
PCR Stage	95	8 sec	45
	60	30 sec*	
*Acquisition step (FAM/VIC Channel)			

F. Instrument compatibility:

The DiAGSure® Dengue TaqMan PCR Kit is compatible with a broad range of real-time PCR platforms. Performance of the kit has been verified on the following systems:
Applied Biosystems® 7500/7500 Fast Real-Time PCR System, Quanstudio 5, Bio-Rad CFX96, Qiagen Rotor Gene Q, Roche Lighcycler® 480.

G. Performance Evaluation:

Specificity:

The target sequences detected in this kit are the conserved regions of DenV cPrM and POLY genes. The primers used in the assay failed to give any amplification from human RNA extracted from control blood and serum eliminating the possibility of non-specific annealing with human DNA.

Furthermore, the primers did not cross-react with other pathogens like Japanese encephalitis virus, Crimean-Congo hemorrhagic fever virus, Yellow fever virus, Murray Valley encephalitis virus, Usutu virus, Marburg virus (MARV), Chikungunya virus,

Ebolavirus, Hepatitis C virus, Lassa virus, West Nile virus, Plasmodium falciparum, and no non-specific amplification was obtained.

Detection limit:

The absolute sensitivities were obtained using a dilution series of quantified synthetic RNA as a template that can be detected with a positivity rate of 95%. The Limit of Detection (LoD) was found to be **8.5 RNA copies/µL** under *in vitro* conditions.

Safety information:

The DiAGSure® Dengue TaqMan PCR Kit is for laboratory use only. Use proper safety measures while handling clinical samples, like wearing mask, gloves, lab-coat, etc.

Ct cut-off for each fluorescent channel:

Target	Cut-off Ct	Interpretation
DenV (FAM)	Ct≤40	Dengue positive
IC (VIC)	Ct≤35	Internal control positive

Interpretation of the results:

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid. If the controls are not valid, the patient results cannot be interpreted.

Refer to the table below for the validity and the interpretation of each specimen result according to the results of each channel.

Target	Dengue	IC	Interpretation	Results
Fluorophore *	FAM/ Green	VIC/Yellow		
Case-1	Positive	Positive/ Negative	DenV positive	Dengue genomic RNA is detected in the clinical specimen.
Case-2	Negative	Positive	DenV Negative	Dengue genome is below the limit of detection of the kit.
Case-3	Negative	Negative	Invalid	Testing should be repeated once from extraction. If a second failure occurs, it is reported to sender as invalid and sample recollection is recommended.

Technical assistance:

Satisfaction of the customers is our utmost priority. For any kind of technical assistance, always feel free to reach out to us at tech.support@gccbiotech.co.in.