DiAGSure™ Dengue genotyping Kit (TaqMan based) v2.0

Catalog No.: GDQ6008- 24R; GDQ6008- 48R; GDQ6008-96R

Kit Components:

Cap color	Reagents	Description	24 Tests (GDQ6008-24R)	48 Tests (GDQ6008-48R)	96 Tests (GDQ6008- 96R)
Red	DiAGPath Master Mix	Enzyme mix	700 µL	1.4 mL	1.4 mL X 2
Amber	DenV1+2 Primer-Probe Mix	Amplification Reagent	50 µL	100 µL	200 µL
Amber	DenV3+4 Primer-Probe Mix	Amplification Reagent	50 µL	100 µL	200 µL
Yellow	DenV1-4 Positive Control (PC)	Positive Control for Dengue 1-4	80 µL	200 µL	400 µL
Blue	Negative Control (NC)	Negative Control	200 µL	500 µL	1 mL
White	RNA Dilution Buffer	Tris-based buffer	500 µL	1 µL	1.5 mL

Intended Use:

The DiAGSure™ Dengue genotyping Kit (TaqMan based) is an in vitro diagnostic (IVD) real-time reverse transcriptase polymerase chain reaction (qRT-PCR) test involving TaqMan chemistry intended for the detection of specific genotype of dengue virus (DenV1/DenV2/DenV3/DenV4) infecting an individual. Serum or plasma samples are mostly used for dengue detection and genotyping studies. However, whole blood samples may also be used. Per sample, two reactions are set up in multiplex PCR. The DenV polyprotein (POLY) gene has been targeted for each genotype. Each reaction entails an endogenous RNase-P internal control (IC) as a measure of sample quality and extraction procedure.

Estimated operating time:

~1 hour 10 minutes

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.
- This product can be used for 30 days after opening the vials.
- This product can be used for maximum 7 repeats of freezing and thawing.

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. Storage condition is 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. If the extracted RNA is not immediately tested after extraction, they can also be stored at -80°C and repeated freezing and thawing should be avoided. It is advised to start amplification with at RNA of high purity (O.D.260/280 = 1.9-2.0). A starting RNA concentration of at least 10 ng/ µL should be used. Too high RNA concentration might necessitate template dilution according to requirement. Note: Use of freshly extracted RNA is recommended.

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 2 parallel PCR reactions (each for a specific genotype) according to the following table:

Component	Volume per reaction	
	Tube-1	Tube-2
DiAGPath Master Mix	13 µL	13 µL
DenV1+2 Primer-Probe Mix	2 µL	--
DenV3+4 Primer-Probe Mix	--	2 µL
Extracted RNA/ Positive Control (PC)/Negative Control (NC)	6 µL	6 µL
RNA dilution buffer	4 µL	4 µL
Total Volume	25 µL	25 µL

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:

Gene	Channel	Source wavelength (nm)	Detection wavelength (nm)
DenV 2/3	FAM (Green)	470	510
DenV 1/4	HEX/VIC (Yellow)	530	555
Reference gene (IC)	Cy5 (Red)	625	660

E. PCR cycling conditions:

Step	Temperature (°C)	Time	No. of cycles
Hold stage 1(RT-step)	50	10 min	1
Hold stage 2	95	3 min	1
PCR Stage	95	10 sec	40
	60	30 sec*	
***Acquisition step (Green/Yellow/Red Channel)**			

F. Instrument compatibility:

The DiAGSure™ Dengue genotyping Kit (TaqMan based) 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, Quantstudio 5, Bio-Rad CFX96, QIAGEN Rotor Gene Q, Roche Lightcycler® 480, Analytik Jena qTOWER G-touch and Himedia Q96-Plus.

G. Performance Evaluation:

- **Specificity:**
  The target sequences detected in this kit specifically targets the polyprotein (POLY) gene of Dengue virus. The primers used in the assay failed to give any amplification from RNA extracted from the blood of healthy individuals eliminating the possibility of non-specific annealing with control human RNA.

  Furthermore, the primers did not cross-react with other flaviviruses like HCV, Zika, Yellow fever virus and JEV as well as the most common bacteria like *E. coli*, *B. subtilis, Pseudomonas* sp. and pathogens like *Mycobacterium tuberculosis, Streptococcus pneumoniae, Vibrio cholerae, Chlamydia trachomatis*, and *Salmonella typhi*.

- **Detection limit:**
  The absolute sensitivities were obtained using a dilution series of quantified synthetic RNA as a template for detection. The Limit of Detection (LoD) was found to be 100 copies of DenV synthetic RNA under in vitro conditions.

- **Safety information:**
  The DiAGSure™ Dengue genotyping Kit (TaqMan based) is for laboratory use only. Use proper safety

measures while handling clinical samples, like wearing mask, gloves, lab-coat, etc.

C_t cut-off for each fluorescent channel:

The Ct cut off for amplification is given below. Sigmoidal amplification curves within the Ct cut-off range indicate positive amplification for a specific DenV genotype.

Target	Cut-off C_t	Interpretation
DenV (1/2/3/4) gene	Ct≤36	DenV (1/2/3/4) positive
IC	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.

Reaction-1

DenV1 (VIC)	DenV2 (FAM)	IC	Interpretation
+	-	+	DenV1 Positive
-	+	+	DenV2 Positive
-	-	+	Negative for DenV1 & DenV2
-	-	-	Invalid

Reaction-2

DenV3 (FAM)	DenV4 (VIC)	IC	Interpretation
+	-	+	DenV3 Positive
-	+	+	DenV4 Positive
-	-	+	Negative for DenV3 & DenV4
-	-	-	Invalid

* If the target gene signal (FAM/VIC Channel) is strong, the IC (Cy5 Channel) may be negative. Absence of IC amplification in FAM/VIC negative samples indicates invalid results. In such cases, testing should be repeated from extraction.

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.