DiAGSure® Human Metapneumovirus (HMPV) Detection Kit

Catalog No.: GDQ6055- 20R; GDQ6055- 50R; GDQ6055-100R

Color Coding (Caps)	Reagents	Description	GDQ6055-20R (20 tests)	GDQ6055-50R (50 tests)	GDQ6055-100R (100 tests)
Red	2X One-step Master Mix	Amplification Mix	300 µL	700 µL	1.4 mL
Amber	Primer-Probe Mix	Amplification Reagent	40 µL	80 µL	200 µL
Yellow	Positive Control (PC)	Positive Control	20 µL	50 µL	100 µL
Blue	Negative Control (NC)	Negative Control	200 µL	500 µL	1 mL
White	RNA Dilution Buffer	Tris-based buffer	200 µL	500 µL	1 mL

Intended Use:

Symptoms commonly associated with HMPV include cough, fever, nasal congestion, and shortness of breath. Clinical symptoms of HMPV infection may progress to bronchitis or pneumonia and are similar to other viruses that cause upper and lower respiratory infections. The estimated incubation period is 3 to 6 days, and the median duration of illness can vary depending upon severity but is similar to other respiratory infections caused by viruses.

The DiAGSure® HMPV detection PCR assay can detect Human Metapneumovirus and is a much more sensitive and fast detection method for the detection of viral infection. The DiAGSure® HMPV Detection Kit (Multiplex, 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 viral RNA in sputum, BAL, tracheal aspirate and oropharyngeal or nasopharyngeal swab in VTM samples. This two-plex PCR detects the nucleoprotein (N) gene of the viral genome and includes an internal control (IC) reference gene in a single-tube reaction. The kit can detect all four lineages of human metapneumovirus—A1, A2, B1 and B2.

Estimated operating time:

~1 hour(s) 20 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.
- 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.

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 at -80˚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 = 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 qRT-PCR protocol:

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

Components	Volume per reaction
2X One-step Master Mix	13 µL
Primer-Probe Mix	2 µL
Extracted RNA/ Positive Control (PC)/Negative Control (NC)	10 µL
Total Volume	25 µL

D. Real-time PCR Instrument set up:

Set up the PCR tubes/strips/plate in the Real time PCR 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)
Nucleoprotein (N) gene	FAM (Green)	470	510
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	45
	58	30 sec*	
***Acquisition step (Green/ Red Channel)**			

F. Instrument compatibility:

The DiAGSure® Human Metapneumovirus (HMPV) Detection 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, Quantstudio 5, BIORAD™ CFX96, QIAGEN® Rotor Gene Q, Roche Light-cycler® 480, Analytik jena qTOWER^3 and HiMedia InstaQ96.

G. Performance Evaluation:

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

  Furthermore, the primers did not cross-react with other viruses like COVID-19, RSV, Rhinoviruses, Influenza virus A & B, Adenovirus, Parechovirus, Bocavirus, Mumps virus, Dengue virus, Chikungunya virus, Hepatitis C virus and Hepatitis B virus. Also, the primers did not cross-react with the most common bacteria like *E. coli*, *B. subtilis*, *Pseudomonas* sp. as well as pathogens like *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, *Vibrio cholerae*, *Chlamydia trachomatis*, and *Salmonella typhi*, and the protozoa *Plasmodium* sp. and *Giardia lamblia*.

- **Detection limit:**
  The absolute sensitivities were obtained using a dilution series of quantified synthetic RNA (*in-vitro* transcribed) as a template for detection. The Limit of Detection (LoD) was found to be 50 RNA copies/reaction under in-vitro conditions.

- **Safety information:**
  The DiAGSure® Human Metapneumovirus (HMPV) Detection Kit 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:**

Target	Cut-off C_t	Interpretation
Viral gene - nucleoprotein (N) gene	Ct≤40	Viral RNA positive
IC (Cy5)	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's result 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	Viral gene	IC	Interpretation	Results
Fluorophore*	FAM/ Green	Cy5/Red		
Case-1	Positive	Positive/ Negative	HMPV Positive	Viral infection is detected in the clinical specimen.
Case-2	Negative	Positive	HMPV Negative	Viral infection is not detected in the sample.
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.

* If the target gene signal (FAM Channel) is strong, the IC (Cy5 Channel) may be negative.

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.