

Read the instruction leaflet carefully prior to testing. For professional use only.



Middle East Respiratory Syndrome (MERS) Real Time RT-PCR Kit

Middle East Respiratory Syndrome (MERS) Real Time RT-PCR Kit for the qualitative/quantitative detection of nucleic acids from Nasopharyngeal swabs, Oropharyngeal Specimens, Serum and Seminal Plasma of infected patients.

INTENDED USE

Middle East Respiratory Syndrome (MERS) Real Time RT-PCR Kit is designed for the qualitative/quantitative detection of nucleic acids from Nasopharyngeal swabs, Oropharyngeal specimens, Serum and Seminal Plasma of infected patients. The results can be used to assist diagnosis of patients with MERS infection and provide molecular diagnostics. The test results of this product are for clinical reference only and should not be used as the only standard for clinical diagnosis. It is recommended to conduct a comprehensive analysis by combining the test results with clinical symptoms and other laboratory tests.

INTRODUCTION

Middle East respiratory syndrome (MERS) is a viral respiratory disease caused by Middle East respiratory syndrome coronavirus (MERS-CoV) that was first identified in Saudi Arabia in 2012. Coronaviruses are a large family of viruses that can cause diseases ranging from the common cold to severe acute respiratory syndrome (SARS) and Coronavirus disease-2019 (COVID-19). Typical MERS symptoms include fever, cough and shortness of breath. Pneumonia is common, but MERS patients may not always develop this condition. Gastrointestinal symptoms, including diarrhea, have also been reported among MERS patients. Approximately 35% of MERS cases reported to WHO have died.

PRINCIPLE

The primer and probe mix targets the specific conserved sequence encoding the *ORF1ab* gene. With the PCR reaction mix provided, the amplification of template can be quantitatively monitored by the increasing fluorescence signal detected by a real-time PCR instrument.

The PCR detection system includes an endogenous internal control primer and probe mix. The result of internal control provides the accuracy of sampling and extraction process, in order to avoid false-negative results.

Middle East Respiratory Syndrome (MERS) Real Time RT-PCR Kit contains amplification reagents, consisting of the following:

Description	Quantity	Storage
1. Test	10 Vials	RT
2. Positive Control	1 Vial	RT
3. Negative Control	1 Vial	RT
4. Resuspension Buffer	100 µl/vial	RT

INSTRUMENT COMPATIBILITY

Middle East Respiratory Syndrome (MERS) Real Time RT-PCR Kit is compatible with the following:

Real Time RT-PCR Instruments with channels
• FAM
• HEX

OTHER MATERIALS APART FROM KIT COMPONENTS, REQUIRED TO PERFORM THE TEST.

- New pair of disposable gloves and facemask
- Biohazardous waste container
- RNA extraction kit

STORAGE AND STABILITY

1. The shelf-life of components is 12 months. Manufacturing date is indicated on the box.
2. Reagents should be stored in dark at Room Temperature.
3. The reconstituted liquid reagent should be used up at once.

SPECIMEN REQUIREMENT

1. Sample Type: Nasopharyngeal swabs, Oropharyngeal Specimens, Serum and Seminal Plasma of infected patients.
2. Sample Collection: Collection accordance with conventional sample collection methods.

3. Sample Storage and Transportation: Sample to be tested can be processed immediately or stored at -20°C ($\pm 5^{\circ}\text{C}$) for 3 months, or -70°C for the long term. Avoid repeated thawing and freezing. Sample should be transported with refrigerant packs in a sealed Styrofoam box or ice chest.

WARNINGS AND PRECAUTIONS

1. This product is to be used only for *in vitro* diagnostic detection. For use only by laboratory-trained professionals. Please read this manual carefully before using it.
2. The contamination of laboratory environment and reagent, or cross-contamination during specimen treatment may lead to a false-positive result.
3. The operation procedure and precautionary warnings of this instrument should be well understood before conducting the test. Quality control should be performed for each test.
4. The decrease of detection effect: A false-negative result may occur if there are any mistakes in the transportation, storage and operation of reagents.
5. Handle all specimens as if infectious, using safe laboratory procedures. All samples should be regarded as potentially infectious materials. Laboratory workers should wear disposable gloves, laboratory coat/gown, etc. Gloves should be changed after handling each sample, to avoid contamination and false results. Laboratory management should be strictly in accordance with the regulations of PCR gene amplification laboratories. Laboratory personnel must be professionally trained, and the experiment process should be strictly divided into sections/organized. All consumables should be properly sterilized and used only once. Instruments and equipment should be assigned to each stage of the experiment and alternative use of the same should be prohibited.
6. Inappropriate sample collection, transfer, storage and operation may lead to inaccurate test results. RNA extraction should be carried out as soon as possible after sample collection to avoid degradation. If it cannot be carried out immediately, it should be stored in accordance with suitable specimen storage procedures. As this test involves the extraction of RNA and PCR amplification, please take care to avoid contamination of the amplification reaction mixture. Also, regular monitoring of laboratory contamination is recommended.
7. When using this kit, please follow the instructions strictly. The collection, storage and transfer of samples, the extraction and detection of RNA, and the interpretation of results must be carried out in strict accordance with the requirements specified in the kit instructions. The processes of sample preparation and addition must be carried out in the biosafety cabinet or other basic protective facilities according to the technical requirements of the regulatory standards.
8. The operation of sample and waste should meet the requirements of relevant laws and regulations. Discard all materials in a safe and acceptable manner, in compliance with all legal requirements. If exposure to skin or mucous membranes occurs, immediately wash the area with large amounts of water. Seek medical advice immediately. Do not use components beyond the expiration date printed on the kit boxes. Do not mix reagents from different lots. Return all components to the storage condition after preparing the working reagents. Do not interchange vial or bottle caps, as cross-contamination may occur. Reagents supplied are formulated specifically for use with this kit. Make no substitutions in order to ensure optimal performance of the kit. Further dilution of the reagents or alteration of incubation time and temperature may result in erroneous or discordant data.

PREPARATION BEFORE TESTING

Please follow user manual instructions to extract RNA from clinical sample using an RNA extraction kit. Extracted RNA can be used directly for PCR detection. Otherwise, keep RNA sample at -70°C if not in use. Avoid repeated thawing and freezing.

Note: This product does not contain RNA extraction kit and is compatible with other commercial kits.

DETECTION METHOD

1. TEST
Take out the test vial and add 20µL of the extracted sample to resuspend the mix. Let it sit for 30 seconds, then gently pipette up and down to ensure thorough mixing. Spin the tubes for 20-30 seconds, then transfer the entire volume into a PCR strip or well for testing.
2. POSITIVE AND NEGATIVE CONTROL
Add 20 µL of resuspension buffer and gently pipette up and down until the mix dissolved completely and spin the tubes for 30 seconds. Avoid generating air bubbles. Wash the wall of the tube by pipetting thoroughly. Aliquot completely to PCR strip or well.

Note: The reconstituted liquid reagent should be used up at once.

3. SETTING UP PCR

- 3.1. Place the tubes on the sample holder in the instrument. Set up the test panel according to the positions of positive control, negative control and test samples.
- 3.2. Select the detection channels as follows:
Select FAM (Middle East Respiratory Syndrome) and HEX (Internal control) channels to detect Middle East Respiratory Syndrome.

3.3. Enter the amplification program commended as below:

	Step	Temperature	Time	Cycle
1	Reverse Transcription	50°C	15 minutes	1
2	Initial Denaturation	95°C	10 minutes	1
3	Denaturation	95°C	15 seconds	40
4	Annealing	58°C	30 seconds	
	Extension & Fluorescence measurement	60°C	30 seconds	
	Cooling	25°C	10 seconds	1

Save the file after making the settings and run the reaction. Please set the fluorescence internal control of the instrument to "None". For example, for ABI series instruments set "Passive Reference" to "None".

4. RESULT INTERPRETATION: Please refer to the user manual of the instrument for setting the following analysis uses ABI series instruments as an example.

- 4.1. After the reaction is completed, the results are automatically saved, and the amplification curves of the detected target RNA and the internal control are analyzed separately.
- 4.2. According to the analysis, the amplification plot will adjust the Start value End value and Threshold value of the Baseline (users can adjust the values according to the actual situation. Start value can be set within 3~15, and End value can be set within 5~20; users can adjust the amplification curve of negative control to make it linear or below the threshold line). Click "Analyze" to perform the analysis and the parameters should meet the requirements mentioned in "Section 5. Quality Control". Lastly, record the qualitative results in the Plate window.

QUALITY CONTROL

Middle East Respiratory Syndrome PCR Negative Control:

FAM channel does not show Ct value or Ct>35.

Middle East Respiratory Syndrome PCR Positive Control:

FAM and Internal Control (HEX) channels shows at Ct≤35

The above requirements must be met at the same time in the same experiment, otherwise, this experiment is invalid and needs to be repeated.

RESULT INTERPRETATION

Positive Threshold

According to the study of the reference value, the Ct reference value for the target gene detected by this product is 35, and the Ct reference value of internal control is 35.

Result Analysis

Internal control (HEX)	Middle East Respiratory Syndrome (FAM)	Conclusion	Remark
Ct≤35	Has amplification curve; Ct≤35	Positive	Report results to the Sender
Ct≤35	No amplification curve/ Ct>35	Negative	Report results to the Sender
Ct>35	-	Invalid; need collecting sample again	Retest

1. First, analyze the amplification curve internal control HEX channel. If Ct≤35, it indicates that the detection is valid, and users can continue the subsequent analysis:
 - a) If a typical S-type amplification curve is detected by the FAM channel, with Ct≤35, it indicates that Middle East Respiratory Syndrome is positive.
 - b) If FAM channel does not detect a typical S-type amplification curve (no Ct), it indicates that Middle East Respiratory Syndrome is negative.
2. If the internal control HEX channel failed to detect Ct or Ct>35, it indicates that the concentration of the tested sample is too low or there is an inhibitory reaction from the interfering substance. Users have to repeat the experiment.
3. For negative samples, the internal control should be positive. If the internal control is negative, the test result of the sample is invalid. The cause should be found and eliminated. Users should redo sampling and repeat the experiment (if the result is still invalid, please contact the manufacturer).

LIMITATIONS

1. The test results of this product are for clinical reference only.
2. Analysis of the possibility of false-positive and negative results:
 - 2.1 Improper sample collection, processing and transportation and low sample concentration may cause false-negative results.
 - 2.2 Variations in the target sequence or sequence changes caused by other reasons may lead to false-negative results.
 - 2.3 Improper reagent storage can lead to false-negative results.
 - 2.4 Other unproven interferences or PCR inhibitors may cause false-positive results.
 - 2.5 Delayed sample processing may cause false-positive results.
 - 2.6 This assay should be performed according to Good Laboratory Practice (GLP) regulation. Operators should strictly follow the manufacturer's instructions in performing the test.

PRODUCT PERFORMANCE

Specificity

The primer and probe provided is designed based on the conserved sequence of the Middle East Respiratory Syndrome *ORF1ab* gene and has a high detection rate of the target gene fragment. The specificity of the kit was checked and this product has no cross-reactions among positive samples of COVID-19 virus, Influenza A virus, Influenza B virus, Respiratory Syncytial virus, Adenovirus, Parainfluenza virus, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Chlamydia pneumoniae*. The negative and positive rates of detecting commercial reference materials were 100%. The observed values for the Sensitivity of the test kit were 95%.

Linear Range

For the determination of the linear range of the Middle East Respiratory Syndrome (MERS) Real Time RT-PCR Kit, a dilution series Middle East Respiratory Syndrome (MERS) RNA ranging from 100000 - 20 IU/ml prepared in replicates.

The linear range of the Middle East Respiratory Syndrome (MERS) Real Time RT-PCR Kit for the quantification of Middle East Respiratory Syndrome (MERS) is 15 - 200000 IU/ml.

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To report any adverse events, write to contact@thegenes4life.com or call us on +91 9778698070



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