Results :

Sensitivity and Specificity of Meril COVID-19 One-step RT-PCR Kit

Meril COVID-19 One Step RT-PCR Kit	SARS CoV-2 real time PCR Confirmed samples			
		Positive	Negative	Total
	Positive	20	0	20
	Negative	0	75	75
	Total	20	75	95

Parameter	Estimate (%)	Lower-Upper 95% CIs
Specificity	100	88-100
Sensitivity	100	83-100

Analytical sensitivity of testing:

Limit of detection (LOD) for the N assay is < 500 RNA copies/ mL.

Warnings and Precautions:

1. This product is only used for *in vitro* detection. Please read this manual carefully before use.
2. Laboratory personnel should be trained and familiar with the operation procedures and precautions of the instrument before the experiment. Quality control should be performed for each experiment.
3. Laboratory management should be strictly in accordance with the regulations of PCR gene amplification laboratories. Laboratory personnel must be professionally trained and the experimental process should be strictly divided into sections. All consumables should be used only once after sterilization. Instruments and equipment should be assigned to each stage of the experiment and cannot be used alternatively.
4. All samples should be regarded as potentially infectious materials. Laboratory workers should wear appropriate personal protective equipment (PPE) which includes disposable gloves, laboratory coat or grown. Gloves should be changed regularly to avoid cross-contamination between samples.
5. Clinical laboratories involving manipulation of potentially infected specimens should be performed in a certified Class II Biological Safety Cabinet (BSC) in a BSL-2 facility. Diagnostic tests should follow standard laboratory practices, including Standard Precautions, when handling potential patient specimens. For laboratory waste, follow standard procedures associated with other respiratory pathogens

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Symbols used on Meril Diagnostics labels:

REF Catalogue No. | LOT Batch No. | Consult instruction for use
Manufacturer | Expiry Date | Caution
Manufacturing Date | Keep Dry | Keep away from direct sunlight
Storage Temperature | Sufficient for | Do not use if box open or damaged
IVD In vitro Diagnostics | EC REP Authorized European Representative in the European Community | European health & safety product label

Meril COVID-19 One-step RT-PCR Kit

Product Code: NCVPCR-01
Pack Size : 32 tests
Product Code: NCVPCR-02
Pack Size : 96 tests

Introduction:

Corona virus belongs to the family of Coronaviridae, in the order of Nidovirales. It is formed by a positive-sense single-stranded RNA, usually appears spherical with a size of 80-120nm and with crown-like spikes on the surface. This large family of virus is commonly circulating among vertebrates, such as camels, cats and bats. Novel corona virus (COVID-19) has been identified as a new strain of corona virus. It can cause viral pneumonia and dyspnea in humans.

Intended Use:

This kit is designed to detect COVID-19 using real time PCR. The results can be used to assist diagnosis of patients with COVID-19 infection, and provide molecular diagnostic basis for infected patients.

The test results of this kit 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 patients' symptoms and other laboratory tests.

Principle:

The primer and probe mix for this kit adopts the dual-target gene design, which targets the specific conserved sequence encoding the ORF 1ab gene and the nucleoprotein N 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.

Kit Components:

This kit contains real time PCR amplification reagents, composed of the following:

Sr. No.	Components	Volume (32 Test)	Volume (96 Test)
1	COVID-19 Enzyme Mix (Lyophilized)	32 tests/ bottle	96 tests/ bottle
2	COVID-19 Primer-Probe Mix	32 µL/vial	100 µL/vial
3	Enzyme Mix Buffer (5×)	130 µL/vial	400 µL/vial
4	COVID-19 PCR Positive Control	30 µL/vial	90 µL/vial
5	COVID-19 PCR Negative Control (DEPC-treated H_2O)	30 µL/vial	90 µL/vial

Meril Diagnostics

For in vitro diagnostic use
Read this pack insert thoroughly before use

Storage and Shelf-life:

1. Shelf-life of reagent kit is 12 months. Manufacture date is indicated on the box.
2. Reagents should be stored in the dark at -20 ± 5 °C .
3. Repeated thawing and freezing should be no more than 10 times.
4. The reconstituted liquid reagent should be used up at once. Leftover reagents should be stored at 4℃ for no longer than 1 week.

Instrument Compatibility:

This kit is compatible with real time PCR instruments with FAM, HEX/VIC, RED/ROX channels.

Sample Requirement

1. Sample Type: Nasopharyngeal, Oropharyngeal swab specimens, throat swabs, serum and virus preservation buffer.
2. Sample Collection: Collect in accordance with conventional sample collection methods
3. Sample Storage & Transportation: Sample to be tested can be processed immediately, or stored at -20 ± 5 ℃. Avoid repeated thawing and freezing. Sample should be transported with refrigerant packs in sealed Styrofoam box or ice chest.

Preparation before Testing:

Please follow manufacturer's instruction to extract virus RNA from clinical sample using 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 an RNA extraction kit, and is compatible with Qiagen/Thermo Viral RNA Kit and any other commercial kits.*

Assay Setup:

1. Reagent Preparation (Perform in Reagent Processing Area)

1.1 Master Mix Preparations:

Take out the components from the box and let it thaw at room temperature until equilibrated. Resuspend the Lyophilized Enzyme Mix in 400 µL Enzyme Mix Buffer. Add 500 µL RNase-free water and gently pipette up and down. Avoid generating air bubbles. Wash the wall of tube by pipetting to prevent lyophilized powder from remaining. Place the tube aside for 30 min.

Note: *The reconstituted liquid reagent should be used up at once. Leftover reagents should be stored at 4°C for no longer than 1 week.*

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1.2 Reaction Mix Preparation:

The recommended sample volume used in the reaction is 5 µL or 10 µL. Refer to one of the columns below to prepare the reaction mix:

1 × Volume Required		
	For 5 µL Sample	**For 10 µL Sample**
Resuspended master mix	9 µL	9 µL
ORF1ab/N/ICON Primer & probe (FAM/HEX/ROX)	1 µL	1 µL
RNase-free water	5 µL	-
Total volume	15 µL	10 µL

※ Multiply the numbers according to the number of tests.

1.3 Aliquot 15 µL (or 10 µL, depending on sample volume) of the above reaction mix into the PCR plate of the chosen PCR platform. Aliquot into wells according to the number of samples to be tested, include one well for the positive control and one well for the negative control. Transfer the reaction mix to Sample Processing Area.

2. Sample Adding (Perform in Sample Processing Area)

2.1 For 5 µL sample: Add 5 µL of the following into the appropriate wells according to plate setup: Sample(s), Positive Control, Negative Control

2.2 For 10 µL sample: Dilute positive control with 5 µL DEPC-treated water to total volume of 10 µL. Add 10 µL of the following into the appropriate wells according to plate setup: Sample(s), Diluted Positive Control, Negative Control

2.3 After adding the samples, cover the lid immediately. Spin down briefly using a centrifuge to remove air bubbles. Transfer the mixture to amplification area.

3. PCR Amplification (Perform in Amplification and Analysis Area)

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 RNA samples.

3.2 Select the detection channels as following:

a) Select FAM (ORF-1ab gene) and HEX (N gene) channels to detect COVID-19 RNA.

b) Select ROX channel to detect internal control.

3.3 Enter the amplification program. Recommended as below:

	Step	Temp.	Time	Cycle
1	Reverse Transcription	50°C	15 min	1
2	cDNA Initial Denaturation	95°C	3 min	1
3	Denaturation	95°C	15 sec	40
4	Annealing, Extension and Fluorescence measurement	55°C	40 sec	
	Cooling	25°C	10 sec	1

Save the file after 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 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 DNA 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, 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 following requirements mentioned in "Section 5. Quality Control". Lastly, record the qualitative results in the Plate window.

5. Quality Control

5.1 COVID-19 PCR Negative Control:

No amplification should be observed in FAM, HEX & Internal Control (ROX) channels before Ct value 35. If a false positive amplification is observed with any channel in the no template control (NTC) reactions, sample contamination may have occurred and repeat testing is recommended.

5.2 COVID-19 PCR Positive Control:

FAM, HEX & Internal Control (ROX) channels Ct ≤ 35

5.3 Internal Control (*R NaseP):*

Internal Control (ROX) channels Ct ≤ 35

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

	Quality Control		
Control	**ORF1ab gene (FAM)**	**Nucleoprotein N gene (HEX)**	**Internal control (ROX)**
Negative Control	No Ct value or Ct > 35.	No Ct value or Ct > 35.	No Ct value or Ct > 35.
Positive Control	Ct ≤ 35	Ct ≤ 35	Ct ≤ 35
Internal Control *(R NaseP)*	-	-	Ct ≤ 35

Note: This assay runs for 40 cycles however for any interpretation, threshold cutoff cycle Ct is 35

Result Analysis:

1. First to analyze the amplification curve of internal control ROX channel. If Ct ≤ 35, it indicates that the detection is valid, and users can continue the subsequent analysis:

a) If a typical S-type (sigmoidal) amplification curve is detected by the FAM and HEX channel, with Ct ≤ 35, it indicates that COVID-19 virus is positive.

b) If FAM and HEX channels do not detect a typical S-type (sigmoidal)amplification curve (No Ct) or Ct > 35, it indicates that COVID-19 virus is negative.

c) If both FAM and HEX channels detect a typical S-type (sigmoidal) amplification curve with Ct ≥ 36 and ≤ 40, sample shall be considered as suspected. User should repeat the experiment. If upon repetition, Ct value appears in the same Ct range, the sample shall be considered presumptive positive.

d) Ct cut-off for each fluorescent channel is provided below in tabular form.

Target	Ct Value	Interpretation
ORF1ab gene (FAM)	Ct≤35	2019-nCov ORF1ab gene positive
Nucleoprotein N gene (HEX)	Ct≤35	2019-nCov Nucleoprotein N gene positive
Internal control (ROX)	Ct≤35	Internal control positive

The validity and the interpretation of each specimen result according to the results of each channel are given below in tabular form.

ORF 1ab gene (FAM)	Nucleoprotein N gene (HEX)	Internal control (ROX)	Results Interpretation	Action to be taken
Positive	Positive	Positive	SARS-CoV-2 Positive	Report results to sender and appropriate health authority.
Positive	Negative	Positive	Suspected. Needs retesting	If again getting N gene Negative, The interpretation is positive
Negative	Positive	Positive	SARS-CoV-2 Negative	If FAM curve is not linear suspect needs retesting. If again getting ORF1ab gene Negative, Possible infection of other corona virus.
Negative	Negative	Positive	SARS-CoV-2 Negative	Report results to sender
Negative	Negative	Negative	Invalid	Sample should be repeated once again with fresh extraction. If a second failure occurs, it should be reported to sender as invalid and sample recollection is recommended if patient is still clinically indicated

Note: If the target gene signal (FAM, HEX) is too strong, the Internal Control (ROX) may be negative.

2. If the internal control ROX 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 positive samples and virus cultures, there is no requirement of the internal control results. 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 retest result is still invalid, please contact the manufacturer.)
4. Determination of grey area results: If the fluorescence signal of a sample has a significant increase in the FAM and HEX channels, but the Ct value is greater than 40, the sample is in the grey area and needs to be re-examined. If the retest result is still in the grey area, it is judged as positive.

Limitations of Detection Methods:

1. The test results of this kit are for clinical reference only. The clinical diagnosis and treatment of patients should be considered in combination with their symptoms, medical history, other laboratory tests and treatment response.
2. Analysis of possibility of false positive & negative results:
   2.1 Improper sample collection, processing & transportation, and low sample concentration may cause false negative results.
   2.2 Variations in the target sequence of the novel corona virus (COVID-19) 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 negative results.
   2.5 Cross-contamination during 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:

Clinical sensitivity and specificity:

1. 20 Nos. of SARS-COV-2 positive samples (equal representation of samples with low medium and high Ct values) and 75 Nos. of SARS-COV-2 negative samples were tested.
2. 10 nos. other virus positive and SARS COV-2 negative samples tested and found no cross-reactivity i.e. human Corona virus, Influenza A virus, Influenza B virus, Respiratory syncytial virus, Adenovirus, Parainfluenza virus, Streptococcus pneumoniae, Haemophilus influenza, Pseudomonas aeruginosa, Pertussis,