SARS-CoV-2(2019-nCOV) Coronavirus and Influenza A/B Virus Multiplex RT-qPCR Detection Kits

User Manual

Cat.Nos.COV00102(50 rxns×25ul)
COV00103(100 rxns×25ul)

Published 15 Feb 2020

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【Product Name】

SARS-CoV-2 Coronavirus and Influenza A/B Virus Multiplex RT-qPCR detection Kits（Four-Colors qPCR）

【Packing Size】

50tests/Kit、100tests/Kit

【Application】

This kit is used for in vitro diagnostics for 2019-Novel Coronavirus (2019-nCoV) infection including suspected case, suspected aggregative diseases and other scenarios. By testing the suspected person's respiratory specimen, serum or blood, the kit can tell if the specimens contain any virus RNA, so it can be used for clinical screening of suspected cases.

【Principles of testing】

By real-time fluorescent PCR amplification technology, the kit uses fluorescent labeled probes and detects the fluorescent strength from the reporter during the amplification to monitor the PCR products quantity. Both detection probes and internal standard probes have fluorescent reporter and quencher. They are differentiated by labels with different colored fluorescent and detected independently at different wave lengths. When the probes are intact, the reporter is near the quencher, so it is inhibited. With PCR amplification, the probes hybrid with the targeted sequence and are degraded due to the 5'-3' exonuclease activity of the polymerase, and the fluorescent reporter and the quencher are separated, so the fluorescent signal can be detected. By presetting the cycle time, every effective cycle will lead to the increase of the fluorescent strength.

Select the 2019-nCoV N gene and E gene as the target amplifying area, design specific primer and fluorescent probes, use FAM label, detect if the specimen contains any 2019 nCoV RNA. Select the 2019-nCoV ORF1ab gene, design specific primer and florescent probes, use ROX label, detect if the specimen contains any 2019-nCoV RNA. Select influenza A/B specific sequence, design specific primer and fluorescent probes, use Cy5 label, detect if the specimen contains any influenza A/B virus RNA. Select other species' sequence (none-human and none-targeted sequence) as internal primer/probe, label with VIC, monitor the whole process.

The kit also contains UNG enzyme anti-contamination system. Its theory is it selectively hydrolyzes Uracil glycoside bond in the single or double chain of DNA that contains dU. This leads to a DNA chain with nucleotide deletion which will further hydrolyzed in alkaline media under high temperature and thus avoid contamination by PCR amplification.

Table1　4 Fluorescent Signals and Target

	Fluorescent signal	Target
1	FAM	2019-nCoV N gene and E gene
2	ROX	2019-nCoV ORF1ab gene
3	Cy5	Influenza A/B specific sequence
4	VIC	MS2 internal standard (RNA Phage pseudo virus)

【Kit Contents】

Table2 Kit Contents

Reagent Name	Quantity (50 tests/pack)	Quantity (100 tests/pack)
1.Negative control(NTC)	200μL×1 vial	200μL×1 vial
2.Positive control(PTC)	200μL×1 vial	200μL×1 vial
3.Internal standard	50μL×1 vial	100μL×1 vial
4.RT-mix	900μL×1 vial	1800μL×1 vial
5.Enzyme mix	150μL×1 vial	300μL×1 vial
6.2019-nCoV primer probe	100μL×1 vial	200μL×1 vial
7.Influenza A/B primer probe	100μL×1 vial	200μL×1 vial

Note: Don't mix components from different batches.

【Storage and shelf life】

1. The kit shall be kept at -20℃~15℃ with protection from light. The shelf life is 12 months
2. The kit shall be transported in foam box with dry ice and the delivery shall be done within 5 days.
3. The expiration date will not change if the kit is opened and stored at the recommended condition, but it shall not exceed 10 freeze-thaw cycles. Avoid repeated freezing and thawing.
4. Production date and expiration date are marked on the outer box.

【Equipment Requirements】

Fluorescent PCR amplifier; Fluorescent PCR amplifier with 4 colors or multi-color fluorescent PCR amplifier such as ABI Prism®7500, Bio-rad CFX96 etc.

【Specimen Collection】

1. Suitable specimen type: upper respiratory specimen (including nasal swabs, nasopharyngeal swabs / aspirates / washes, and sputum) and lower respiratory specimen (including respiratory aspirates, bronchial washes, bronchoalveolar lavage fluids, and lung biopsy specimens).

2. For detailed methods of specimen collection, please refer to the protocol in the "Microbiology Specimen Collection Manual".

3. The collected specimen should be used for detection within the same day. Otherwise, please store the specimen as follows:

Store at 2℃ - 8℃ for no more than 24 hours;

Store at < -20℃ for no more than 10 days;

Store at < -70℃ for long-term, avoiding repeated freeze-thaw cycles.

4. The specimen should be transported using sealed foam box with dry ice.

【Nucleic Acid Extraction】

1.Performance of rRT-PCR amplification based assays depends on the amount and quality of sample template RNA. RNA extraction procedures should be qualified and validated for recovery and purity before testing specimens.

2.Commercially available extraction procedures that have been shown to generate highly purified RNA when following manufacturer's recommended procedures for sample extraction include: bioMérieux NucliSens® systems, QIAamp® Viral RNA Mini Kit, QIAamp® MinElute Virus Spin Kit or RNeasy® Mini Kit (QIAGEN), EZ1 DSP Virus Kit (QIAGEN), Roche MagNA Pure Compact RNA Isolation Kit, Roche MagNA Pure Compact Nucleic Acid Isolation Kit, and Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit, and Invitrogen ChargeSwitch® Total RNA Cell Kit.

3.Retain residual specimen and nucleic extract and store immediately at -70℃

4.Only thaw specimen extracts that will be tested in a single day. Do not freeze/thaw extracts more than once before testing.If the extracted RNA is not used for detection immediately, please store the RNA at below -70℃, avoiding repeated freeze-thaw.

【Test Procedure】

1 Pretreatment of specimen:

1.1 It's recommended to use nucleic acid extraction kits such as bioMérieux NucliSens® systems, QIAamp® Viral RNA Mini Kit, QIAamp® MinElute Virus Spin Kit or RNeasy® Mini Kit (QIAGEN), EZ1 DSP Virus Kit (QIAGEN), Roche MagNA Pure Compact RNA Isolation Kit, Roche MagNA Pure Compact Nucleic Acid Isolation Kit, and Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit, and Invitrogen ChargeSwitch® Total RNA Cell Kit.

1.2 add 1uL/test internal standard into sample lysate. If only monitor RT-PCR process, just add 0.5uL/test into the extracted RNA.

1.3 Positive control and Negative control don't need to be extracted.It can be added directly to the reaction.

2 Set up reaction mixture

Note: Plate set-up configuration can vary with the number of specimens and work day organization. NTCs and PTCs must be included in each run.

2.1 In the reagent set-up room clean hood, place RT-mix and primer/probes on ice or cold-block. Keep cold during preparation and use.

2.2 Thaw RT-mix prior to use.

2.3 Mix RT-mix and primer/probes by inversion 5 times.

2.4 Briefly centrifuge RT-mix and primers/probes and return to cold block.

2.5 Label one 1.5 mL microcentrifuge tube.

2.6 Determine the number of reactions (N) to set up per assay. It is necessary to make excessive reaction mix for the NTC, PTC, and for pipetting error. Use the following guide to determine N:

- If number of samples (n) including controls equals 1 through 14, then N = n + 1
- If number of samples (n) including controls is 15 or greater, then N = n + 2

Calculate the amount of each reagent to be added for each reaction mixture (N = # of reactions).

Component	Volume（μL）
RT-mix	Nx 9
Enzyme mix	Nx 1.5
2019-nCoV primer probe	Nx 1
Influenza A/B primer probe	Nx 1
Total volume	Nx 12.5

2.7 After addition of the reagents, mix reaction mixtures by pipetting up and down. Do not vortex.

2.8 Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.

2.9 Set up reaction strip tubes or plates in a 96-well cooler rack.Dispense 12.5 μL of each master mix into the tubes.

2.10 Pipette 12.5 μL of NTC into the NTC sample wells. Securely cap NTC wells before proceeding.

2.11 Cover the entire reaction plate and move the reaction plate to the specimen nucleic acid handling area.

3 Template Addition

3.1 Gently vortex RNA nucleic acid sample tubes for approximately 5 seconds.

3.2 After centrifugation, place extracted nucleic acid sample tubes in the cold rack.

3.3 Carefully pipette 12.5 μL of the first sample into all the wells labeled for that sample.Keep other sample wells covered during addition. Change tips after each addition.

3.4 Securely cap the column to which the sample has been added to prevent cross contamination and to ensure sample tracking.

3.5 Change gloves often and when necessary to avoid contamination.

3.6 Repeat steps #3.3 and #3.4 for the remaining samples.

3.7 Cover the entire reaction plate and move the reaction plate to the positive template control handling area.

3.8 Pipette 12.5 μL of PTC into the PTC sample wells.

3.9 Put the entire reaction plate at room temperature (15°C~25°C) for 5 minutes, then transfer the reactions to the PCR device,then cycle according to these guidelines:

Step		Temp	Time	Cycles
1	UNG Incubation	25°C	2min	1
2	RT incubation	50℃	10min	1
3	Enzyme activation	95℃	2min	1
4	Denature	95℃	5sec	45
	Annealing, extension, detction*	60℃	35sec	
*Fluorescent test in extension: Channel for 2019-nCoV E gene and N gene is FAM; Channel for 2019-nCoV ORF1ab gene is ROX; Channel for influenza A/B is Cy5; Channel for internal standard is VIC;				

4 Data Analysis

4.1 description of amplification characteristics cure: it is generally S-shaped, and can be divided into baseline period, exponentially growth period, linear growth period and plateau period, as shown in chart 2。

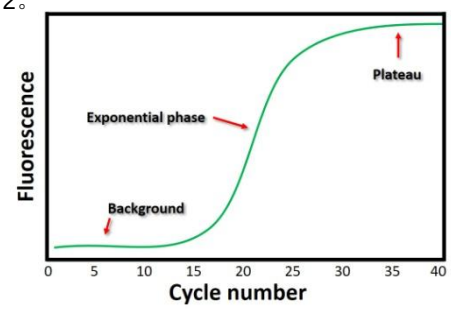

Chart2

4.2 NTC should be negative and not exhibit fluorescence growth curves that cross the threshold line.

If a false positive occurs with one or more of the primer and probe NTC reactions, sample contamination may have occurred.

Invalidate the run and repeat the assay with stricter adherence to the procedure guidelines.

4.3 PTC reaction should produce a positive result with an expected Ct value for each target included in the test.

If expected positive reactivity is not achieved, invalidate the run and repeat the assay with stricter adherence to procedure guidelines.

Determine the cause of failed PTC reactivity, implement corrective actions, and document results of the investigation and corrective actions.

Do not use PTC reagents that do not generate expected result.

4.4 Internal standard should be positive at or before 35 cycles for all clinical samples, thus indicating the presence of sufficient nucleic acid from human RNase P gene and that the specimen is of acceptable quality.

Failure to detect Internal standard in any of the clinical samples may indicate:

Improper extraction of nucleic acid from clinical materials resulting in loss of nucleic acid or carry-over of PCR inhibitors from clinical specimens

Absence of sufficient human cellular material in sample to enable detection

4.5 When all controls exhibit the expected performance, a specimen is considered negative if all 2019-nCoV markers (Fam, Rox) cycle threshold growth curves DO NOT cross the threshold AND the Internal standard(Vic) growth curve DOES cross the threshold line.

4.6 When all controls exhibit the expected performance, a specimen is considered positive for 2019-nCoV if all markers (Fam,Rox) cycle threshold growth curve crosses the threshold line. The Internal standard(Vic) may or may not be positive as described above, but the 2019-nCoV result is still valid.

4.7 When all controls exhibit the expected performance and the growth curves for the 2019-nCoV markers (Fam,Rox) AND the Internal standard(Vic) DO NOT cross the cycle threshold growth curve, the result is invalid. The extracted RNA from the specimen should be re-tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the re-tested sample is negative for all markers and all controls exhibit the expected performance, the result is “Invalid.”

Diagnostic Results

Ct Value*	Result	Recommendations
Ct（FAM）≤36.5 and Ct（ROX）≤38	2019-nCoV positive(+)	Active treatment
Ct（FAM、ROX）≥39 or no value，and Ct（VIC）＜40	2019-nCoV negative(-)	More Rest
Ct（FAM、ROX）≥39 or no value，and Ct（VIC）＜40,but Ct(Cy5) ≤36.5	2019-nCoV negative(-) Influenza A/B positive(+)	Active treatment
Ct（FAM）≤36.5 and Ct（ROX）≤38,and Ct(Cy5) ≤36.5	2019-nCoV negative(+) Influenza A/B positive(+)	Active treatment

only Ct（FAM）≤36.5 or Ct（ROX）≤38	Inconclusive Result	Repeat extraction and double confirm. After getting Ct（FAM）≤36.5 or Ct（ROX）≤38, the result is positive
36.5＜Ct（FAM）＜39 or 38＜Ct（ROX）＜39	Inconclusive Result	Increase specimen concentration and double confirm. If Ct（FAM）＜39 and Ct（ROX）＜39, it means 2019-nCoV result is positive; other results need be analyzed by other methods.
Ct（FAM、ROX、CY5）≥39 or no value，and Ct（VIC）≥40 or no value	Invalid Result	Indicates that there might be issues with experiment itself, reagent, need check before repeat the experiment

*FAM channel is for 2019-nCoV E gene and N gene.

ROX channel is for 2019-nCoV ORF1ab gene.

Cy5 channel is for influenza A/B.

VIC channel is for internal standard .

【Reference value】

After the test negative sample and near the minimum detection limit sample, the reference value of the test result of the target gene (FAM channel) of the kit is determined to be 36.5, and the reference value of the target gene (ROX channel) test result is 38.

【Assay Limitations】

1. Negative result can't completely rule out the presence of the target gene. Below items may influence the assay: low virus content in the specimen, degradation of the specimen, contamination of the specimen, overdose of interfering material in the specimen, incorrect storage of the sample, incorrect storage of the kit, and expiration of the kit etc.
2. The kit offers mainly a qualitative test on the serum and nasopharyngeal swab etc, which is not the exact virus quantity in the original body.
3. The kit is used on target sequence that is highly reservative and stable between strains, but if the target sequence has mutagenesis, it may give psudo-negative result.
4. Specimens can't be detected if its content is below ND limit, so the result shall not be the only test. Need consider the patient's other symptoms, history and other test results.
5. Theoretically nucleic acid method can't completely detect out infection during "window period".
6. The method only applys to the above defined specimen types and test system.
7. If the result conflicts with clinical diagnostics result, it is proposed that double check is needed and the clinical result shall be taken.

【Properties of the kit】

1. Appearance

The package should be tidy, the label is intact, components label is correct and not falling off, all components are well sealed without leaking. The reagent shall be transparent and no suspension

can be seen with naked eyes.

2. **Accuracy**

   Cross referencing with national standard positive products, the conformity is 100%.

3. **Specificity**

   Cross referencing with national standard negative products, the conformity is 100%.

4. **Detection limit**

   Cross-referencing with national standard products with lowest detection limit (10 copies), 3 times, all results are positive.

5. **Repeatability**

   Cross-referencing with national standard products, repeat 10 times, all result are positive and Ct value is CV≤5%.

6. **Anti-Interference**

   The kit won't be influenced by the below products: interferon, nasal spray, nasal drop, steroid, mucoprotein, Zanamivir,Libavelin, Ostavir, Paramive, mobin, tobumycin, hemoglobin, triglycerides, ethanol etc.

7. **Cross reaction**

   The kit doesn't react with human cell system gDNA, local human corona virus such as HKU1, OC43, NL63, H1N1, H3N2, or influenza B.

【Note】

1. The kit is only intended to be used in vitro test. Please read this description before usage and prepare the required reagent and instrument.
2. The kit shall be used only by trained professionals.
3. The kit's result may be influenced by below factors such as the resources of the specimen, the collection of specimen, the quality of specimen, transportation, preparation, DNA extraction, operation, and environment etc, so the result might be pseudo-positive or pseudo-negative, so the user of this kit need to consider this potential risk and limit.
4. The components in the kit are all made per special procedure, so its contents should not be used interchangeably with each other or between the batches.
5. The storage conditions shall not be changed after being opened. Avoid unnecessary freeze-thaw process.
6. The kit contains certain risk and need be handled by the professionals. The operation and disposal of the kit need comply with related regulations either from local, national or international regulations.
7. Need avoid contamination from outside, for example, designated pipette need be used.
8. The result is only for clinical reference. Respective patient's treatment decision need be combined with their diagnostics, history and other test results.
9. The disposal of experiment waste such as the pipette, amplification products etc need be done after harmless treatment.
10. After experiment, use 10% hypochlorous acid or 75% alcohol to decontaminate pipette and the surface of the centrifugal machine, then put it under UV light for 25~30 minutes.

The key properties:

1. High sensitivity 500 copy/ml
2. Very specific, can detect 2019-nCoV E gene, N gene and ORF1ab different zone sequence at the same time in one tube.
3. Can simultaneously identify and distinguish influenza viruses to avoid misdiagnosis.
4. One-step RT-PCR, strong amplification signal, high stability.

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