

**COVID-19 mutation Multiplex RT-PCR detection kit (Lyophilized)
User Manual**

【PRODUCT NAME】

COVID-19 mutation Multiplex RT-PCR detection kit (Lyophilized)

【SIZE】

48tests/kit, 50tests/kit

【INTENDED USE】

The COVID-19 is a Single-stranded RNA Virus ,which Genome mutations are more frequent . The main mutation strains in the world are British B.1.1.7 and South African 501Y.V2 variants. New mutants strain have more obvious transmission power, it necessary to conduct distinguishing diagnostic of COVID-19 mutants for patients.

This kit is intended to in vitro qualitatively detect 4 targets of 2019-nCoV in the throat swab and nasopharyngeal swab samples, covering the main mutation sites of B.1.1.7 mutant strain and 501Y.V2 mutant strain. This kit can simultaneous detect the N501Y, HV69-70del, E484K mutation sites and the new coronavirus S gene. This product is only limited to auxiliary diagnosis of relevant cases during the outbreak of COVID-19.

【PRINCIPLE OF DETECTION】

This product is a fluorescent probe-based Taqman RT-PCR assay system. Firstly, the RNA of 2019-nCov will be reverse transcribed into cDNA by reverse transcriptase, and then PCR amplification will be performed with cDNA as template. During amplification of the template, the Taqman probe will be degraded due to the 5'-3' polymerase activity and exonuclease activity of Taq DNA polymerase, then the separation of fluorescent reporter and quencher enables the fluorescent signal to be detected by instrument. The common N501Y mutation of COVID-19 variants will be detected by FAM channel, the E484K mutation of 501Y.V2 South African mutant strain will be detected by ROX channel, the HV69-70del mutation of B.1.1.7 mutant strain will be detected by VIC channel, and S gene of COVID-19 will be detected by CY5 channel as a reference.

【PRODUCT CONTENTS】

Components	Package	specification	Ingredient
COVID-19 mutation Mix	1 ×bottle (Lyophilized)	50 Test	dNTPs, MgCl ₂ , Primers , Probes,Reverse Transcriptase,Taq DNA polymerase
	6×0.2ml 8 well-strip tube (Lyophilized)	48 Test	
Positive Control	2×0.2ml tube (lyophilized)	8 Tests	Plasmid or Pseudovirus containing N501Y, E484K, HV69-70del mutations and S gene specific fragments
Dissolving solution	1.5 ml Cryotube	800uL	/
Negative Control	1.5 ml Cryotube	200uL	0.9%NaCl

Note: Do not mix the components from different batches for detection. The positive control is Plasmid or Pseudovirus constructed artificially, and they were not infectious.

【STORAGE & SHELF LIFE】

- (1) The kit can be transported by Normal transport.
- (2) All kit components can be stored at 2°C~30°C with protection from light. And the kit is stable for 12 months when stored at the recommended condition.
- (3) See label of outside box for production date and expiration date.
- (4) The lyophilized reagent should be stored at -20°C after dissolution and the repeated freeze -thaw should be less than 4 times.

【INSTRUMENTS】

Our recommendation for platform to use New Coronavirus Variants Detection Kit: Real-time PCR instrument-- Roche LightCycler 480 、 Life Technologies 7500 、 Molarray MA-688, Analytikjena qTOWER serials, and other real-time fluorescence PCR instruments with FAM, VIC, ROX, CY5 channels.

【SAMPLING & HANDLING】

(1) Throat Swab: Use the plastic rod swab with polypropylene fiber head to wipe the bilateral pharyngeal tonsils and the posterior pharyngeal wall at the same time, immerse the swab head into the tube containing physiological saline or VTM, discard the tail, and tighten the tube cover.

(2) Nasopharyngeal swab: Use the Nasopharyngeal flocking swab to collect the sample correctly through

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nasal cavity, immerse the swab head into the tube containing physiological saline or VTM, discard the tail, and tighten the tube cover..

(3) Bronchoalveolar Lavage: Collect bronchoalveolar lavage correctly for test.

The collected sample should be used for detection as soon as possible. If the sample need to be transferred cannot be detected immediately, please store it at low temperature.

The sample can be stored for 24 hours at 2~8°C and for a long time below -70°C. It can also be stored in refrigerator at -20°C temporarily.

Samples shall be transported at low temperature in accordance with biosafety regulations.

【PROTOCOL】

1. Reagent Preparation

a) Lyophilized in bottle version kit: Add 750uL dissolving solution to the bottle to dissolve the lyophilized powder. Divide 15uL of the dissolved reagent into each PCR reaction tube.

b) Lyophilized 8-well strip version kit: Add 15uL dissolving solution to each tube well to resolve the PCR Mix.

c) Positive Control : Add 20uL dissolving solution to one tube well of positive control to resolve it.

Shock and centrifuge them at low speed. The dissolved reagent and positive control can be temporarily stored at 4°C for later use.

*Notes: When using the lyophilized in bottle version kit, after dissolving the reagent can be stored at -20°C and repeated freeze -thaw should be less than 4 times.

2. RNA Extraction

Extract the nucleic acid(RNA) from the specimen using appropriate nucleic acid extraction kit and following the instructions of extraction kit.

After RNA extraction, the extracted RNA shall be added to the PCR reaction tubes within 15 minutes, or transferred to the centrifuge tubes and stored at -15 °C~-25 °C.

3. Template Addition

Add 5 μL Negative Control, 5 μL Positive Control, and Add 5 u L extracted nucleic acid of each specimen into each PCR reaction tube. Shock and centrifuge them at low speed. Then, move them to the Real-time PCR instrument.

4. PCR Amplification

Recommended Setting

Step	Temperature (°C)	Time	Cycle	
1	Reverse Transcription	50	10mins	1
2	Pre-denaturation	95	2mins	1
3	Denaturation PCR	95	10s	40
4	annealing/extension	60	20s	

*Note: The signals of FAM, VIC, ROX and CY5 fluorescence channels will be collected at 60°C. Select "None" for the passive reference on operation interface of ABI7500 PCR software.

5. Data Analysis (ABI7500)

Test data file need to be saved after PCR reaction. Please set the parameters and analysis the results of FAM, VIC, ROX and CY5 channels respectively.

(1) Baseline setting: the baseline can be set automatically or adjusted according to the shape of amplification curve.

(2) Threshold setting: the threshold value should be higher than the highest fluorescence value of negative control in this kit.

6. Quality Control

Negative control and positive control provide the quality control for the assay and shall be conducted for each run of test. The result is valid if ALL the below criteria is met. Otherwise, the test is invalid. In this case, the errors of instruments, reagents, amplification conditions, etc. shall be checked, and the experiment shall be repeated.

Products of Quality Control	Requirements of Quality Control			
	FAM Channel	VIC Channel	ROX Channel	CY5 Channel
Positive Control of New Coronavirus Variants	Ct ≤ 32	Ct ≤ 32	Ct ≤ 32	Ct ≤ 32
Negative Control	Undet	Undet	Undet	Undet

7. Interpreting Test Results