Multiple Real-Time PCR kit for Detection of 2019-nCoV

【Product name】 Multiple Real-Time PCR kit for Detection of 2019-nCoV

【Package specification】 48 Tests

【Intended use】

The kit is used for the qualitative detection New coronavirus (2019-nCoV) ORF1ab and N genes of pneumonia suspected cases of new coronavirus infection in throat swabs and sputum samples in vitro.

The assay is used for the auxiliary diagnosis of the relevant cases and the emergency reserve of the epidemic in vitro during the period of pneumonia infected by the novel coronavirus (2019-nCoV). The novel coronavirus pneumonia diagnosis and novel coronavirus infection control plan should be followed.

The detection of the novel coronavirus should be in line with the requirements of technology guide of novel coronavirus pneumonia laboratory testing from China CDC and so on, and the Biosafety is the most important.

【Principle of Procedure】

In using this kit, the samples are treated with compound nucleic acid lysis buffer, which integrates nucleic acid lysis, RNase inhibition and RNA protection function together to achieve the "one-step" RNA detection. In this kit, RNA reverse transcription reaction and polymerase chain reaction (PCR) combined with TaqMan technology are used to amplify the target nucleic acid fragments with the specific primers designed according to the nucleic acid sequence of the virus. At the same time, the highly specific TaqMan probe can be combined with the target nucleic acid fragments, and will be hydrolyzed to produce the fluorescence signals under the action of reverse transcriptase /Taq enzyme exonuclease activity, in which the real-time amplification curve will be obtained according to the relationship between the fluorescence signal and the number of amplification cycles, besides, the false negative and the PCR inhibition can be monitored by Internal Control.

The kit of 2019-nCoV nucleic acid can be confirmed after the ORF1ab gene (FAM channel) and N gene (Cy5 channel) of 2019-nCoV being qualitatively detected under the quality control by internal control (VIC channel).

【Components】

1. Sample Preservation Solution

ID	Component Name	Specification
1	Sample Preservation Solution	700μL ×48

Storage conditions: 2-30℃

2. Reagent Component

ID	Component Name	Specification
1	RT-PCR Reagent	1.70mL×1
2	Enzyme Mixture	100μL ×1
3	Negative Control	110 μL×1
4	Positive Control	110 μL×1
5	Lysis Buffer (Internal Control inside)	300 μL×1

Remarks:

1. The different batches are not recommended to be mixed.
2. The Enzyme Mixture should be centrifuged at low speed for several seconds before using.
3. Required but not provide: Throat swab, centrifuges, pipettes with suitable measuring range and tips, etc.

【Storage and Expiration Date】

1.Storage conditions: Reagent Component should be stored at - 20 ± 5 ℃ in dark.
2.Validity: tentatively 6 months.
3.The production date and expiration date are shown in the outer package.
4.The repeated freezing and thawing of the kit should not exceed 3 times.

【Applicable Platforms】

The kit can be used on SLAN, ABI 7500

【Sample Requirements】

1. Specimen Type: Throat swab and Sputum

Remarks: The purified nucleic acids with commercial kit can also be used in this kit.

2. Specimen Collection:

Throat swab: Wipe both tonsils and posterior pharyngeal wall with swab at the same time, break off the swab head along the crease into the sample preservation solution tube. Discard the tail, and tighten the tube cover.

Sputum: Dip the swab in to the sputum, then break off the swab head along the crease into the sample preservation solution tube. Discard the tail, and tighten the tube cover.

3. Specimen Storage:

Stored samples at 2℃ ~ 8℃for no more than 24 hours, at - 20℃ if it's over 24 hours, or at -70℃ for a long time.

4. Specimen transport: Transport the specimen in sealed container on ice, and the transportation shall comply with the relevant national biosafety regulations on class II pathogens.

【Protocol】

Please read the following procedure carefully before starting.

1. PCR Reagent preparation (performed in Reagent Preparation Area)

1.1 Thaw RT-PCR Reagent in dark at room temperature, Vortex and centrifuge instantaneously.

1.2 PCR mix preparation: PCR reaction mixture are prepared according to the proportion of 33μL [RT-PCR Reagent] and 2μL [Enzyme mixture] for each test.

2. Specimen preparation and PCR Setup (performed in Specimen Preparation Area)

2.1 Thaw the negative control, the positive control, the specimen to be tested or the purified nucleic acid with commercial kit at room temperature.

SPECIAL NOTE: THE SAMPLE MUST BE VORTEXED AND CENTRIFUGED AT LEAST FOR 1 TO 2 MINUTES AT 13000RPM.

2.2 Add 5μl Lysis buffer into the bottom of each PCR tube, then add 10μl of the negative control, the positive control, the specimen to be tested or purified nucleic acid with commercial kit respectively, gently mix it for 2-3 times with pipette, then leave it at room temperature for 5 minutes.

2.3 Add 35μl prepared PCR mix into each tube, cover it and mix it upside down, then centrifuge instantaneously, to be ready for PCR amplification.

3. PCR amplification (performed in PCR Amplification and Data Analysis Area)

1) Place the PCR tubes into the machine, and the cycle parameter setting is as below:

Step	Temperature	Incubation Time	Cycles
1	50℃	15min	×1
2	94℃	3min	×1
3	94℃	10sec	×42 （collect signal at 58℃）
	58℃	35sec	

2) The reaction volume is 50μL, and the selection of fluorescence channel is as below:

Fluorescence channel	A (ORF1ab gene)	B (Internal Control)	C (N gene)
Fluorescence	FAM	HEX/VIC	Cy5

4. **Baseline and Threshold**

The baseline is usually setup by the software automatically. Baseline adjustment principle: Select the region with relatively stable fluorescence signal before exponential amplification, and avoid the signal fluctuation in the initial stage of fluorescence acquisition at the start, then reduce the CT value of the end by 1-2 cycles compared with the sample with the earliest exponential amplification. The threshold setting principle is that the threshold line just exceeds the highest point of the fluorescence curve of negative quality control.

5. Quality Control

Under the situation that all quality controls are normal, the results are:

Quality control	Fluorescence channel	Normal result (CT value)
Negative control	A (ORF1ab gene)	No numerical value
	C (N gene)	
	B (Internal Control)	With S-type amplification curve, CT < 39
Positive control	A + C (ORF1ab gene and N gene)	CT ≤39 with S-type amplification curve

6. Results judgment

According to the information of the detection results, the specimen's final result is:

Channel			Results judgment
A（FAM）	B（VIC）	C（CY5）	
Ct≤39	Ct＜39	Ct≤39	2019-nCoV positive
No Ct or Ct＞39	Ct＜39	No Ct 或 Ct＞39	2019-nCoV negative
No Ct or Ct＞39	Ct＜39	Ct≤39	Retest*
Ct≤39	Ct＜39	No Ct or Ct＞39	

* It is suggested to collect sputum samples for retest. If the retest results of channel A and/or channel C persist in positive or CT≤ 39, the results are positive, otherwise it is negative.

【Limitations of the test】

1. The test results of this kit should be combined with other relevant medical examination results for comprehensive analysis, and should not be used as the diagnosis basis alone.
2. Unreasonable collection, transportation and storage of specimen, improper operation and improper laboratory environment may lead to inaccurate results.
3. Other unverified interference or PCR inhibitors may result in false negative results.
4. The mutation of 2019-nCoV sequence or sequence changes caused by other reasons may result in false negative results.

【Product Performance】

1. The minimum detection limit of this kit: 2 × 102 Copies / ml.
2. There is no cross reaction with other common respiratory viruses such as Human coronavirus HKU1, SARS coronavirus, MERS coronavirus, Human coronavirus OC43, Human coronavirus NL63, Human coronavirus 229E, influenza A virus(H1N1, H3N2, H5N1, H7N9) , influenza B virus (Yamagata,Victoria), respiratory syncytial virus(RSV-A, RSV-B), Parainfluenza virus (HPIVs 1, HPIVs 2, HPIVs 3), Rhinovirusm (RhV-A, RhV-B, RhV-C), Adenovirus (Ad1, Ad 2, Ad 3, Ad 4, Ad 5, Ad 7, Ad 55) and Human metapneumovirus.
3. The test results of the negative reference of the enterprise should be negative.
4. The test results of the positive reference of the enterprises should be positive.
5. The LODs were repeated 20 times, and the detected rate should be ≥ 90%.
6. The samples with high and low concentrations were detected 10 times respectively, and the Coefficient of variation of Ct value (CV,%)≤5%.

【Precautions】

Please read this manual carefully before testing.

1. If a commercial nucleic acid extraction kit is used, the purified nucleic acid should also be mixed well with 5μl lysis buffer in kit, then proceed to the next step.
2. If the sample collection tube recommended of this kit is not used, the collected samples shall be verified. The verification method: Use the collection solution as the diluent to dilute the nucleic acid of the confirmed high concentration specimen by 10 times, at the same time, the water with RNAse free will work as the diluent control. If the result is good, it can be used.
3. For the external quality control with preservatives or for the collection tubes with interference, it is recommended to use commercial kit to extract nucleic acid firstly.
4. The whole detection process should be strictly divided into three areas: Reagent Preparation Area, Specimen Preparation Area, PCR Amplification and Data Analysis Area. Instruments, equipment, consumables and work clothes in each area must be used independently and exclusively. After the experiment, the working table must be cleaned and disinfected.
5. Use disposable gloves (often replaced) without fluorescent substances, disposable special reaction pipe, self-unloading liquid pipette and suction head with filter tip.
6. Biosafety cabinet (negative pressure type) or anti-pollution cover must be used for sample preparation to prevent environmental pollution.
7. Negative and positive quality control must be set for each experiment.
8. Operators must be trained professionally and have certain experience and operation skills.
9. Instruments and equipment such as operation platform, pipette, centrifuge and PCR amplification instrument shall be disinfected with 0.5% hypochlorite or 75% alcohol, ultraviolet lamp or ozone frequently.
10. Samples in the experiment, waste materials (such as tips) that have been in contact with quality control, and centrifuge tubes after amplification should be considerated infectious, these materials must be treated as medical wastes only after they are detoxified.

【Basic Information】

Name of registrant / Manufacturer: Beijing NaGene Diagnosis Reagent Co., Ltd.
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Production license No. of medical device: Beijing Food and Drug Administration production license No. 20180019

【Medical device registration certificate No./Product technical requirements No.】

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