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SARS-CoV-2 (2019-nCoV) ORFlab/N/E Genes Triple Dye-Based Quantitative RT-PCR Kit

Product Number: DTK564

Shipping and Storage

Low temperature transportation, stored at -20℃, with a shelf life of 12 months. Positive controls need to be placed separately due to their tendency to contaminate other components. This product does not provide live samples as positive controls, only nucleic acid fragments as positive controls.

Component

Component	Specification	Nine hole box packaging
2 × qRT-PCR buffer	500μL	Brown cover
10 × qRT-PCR enzyme mixture	100μL	Red cover
Fluorescent PCR specific template diluent	1mL	Green cover
Novel coronavirus triple dye qRT-PCR primer mixture	360μL	White cover
Novel coronavirus triple dye qRT-PCR positive control (1 × 10E7 copies/μL)	90μL	Yellow cover

Description

This kit can quickly detect three genes of ORFlab gene/N gene/E gene of novel coronavirus (2019 nCoV) in a single reaction.

Application

This product is developed based on the principle of dye based fluorescent quantitative PCR, and is a special kit for detecting the triple of novel coronavirus. It has the following characteristics:

1. One stop shop, users do not need to prepare each ingredient separately, including primers and controls.
2. The primers designed according to the conserved gene sequence of the triple genome of novel coronavirus have good specificity.
3. Based on dye based qRT-PCR detection, the sensitivity is 10-100 times higher than conventional RT-PCR.
4. Using one tube qRT-PCR technology, RT and PCR are completed in one tube without the need for intermediate sample transfer, reducing operational errors and potential contamination.
5. This product is sufficient for 50 rounds of RT-PCR in a 20μL system and can only be used for scientific research, not for clinical use..

Specimen collection

Sample RNA.

Protocol

1. **Dilute the positive control (taking the 10 fold dilution of the 6 samples 10E1-10E6 as an example. Due to the high concentration of the standard, the following dilution operations must be performed in separate areas). To increase product stability and avoid the spread of infectious pathogens**
   1.1. Mark 6 centrifuge tubes, namely 6, 5, 4, 3, 2, and 1. Add 45μL of fluorescent PCR specific template dilution solution using a core gun tip, preferably using a core gun tip. The same applies below.
   1.2. Add 5μL of 1 × 10E7 copy/μL positive control (provided in this kit) to tube 6, shake thoroughly for 1 minute, and obtain 1 × 10E6 copy/μL positive control. Put it on ice for later use.

1.3. Change the gun head and add 5μL of 1 × 10E6 copy/μL positive control (obtained from the previous dilution) to tube 5. Shake thoroughly for 1 minute to obtain 1 × 10E5 copy/μL positive control. Put it on ice for later use.

1.4. Change the gun head and add 5μL of 1 × 10E5 copies/μL positive control (obtained from the previous dilution) to tube 4. Shake thoroughly for 1 minute to obtain 1 × 10E4 copies/μL positive control. Put it on ice for later use.

1.5. Repeat the above operation until 6 dilutions of positive controls are obtained. Put it on ice for later use.

2. Preparation of Sample RNA

2.1. If there are N samples, N+2 extractions must be set, with the additional one being PC (positive control for sample preparation) and one being NC (negative control for sample preparation). A positive control can be prepared by adding a certain amount of water to the fourth sample (with a concentration of 1 × 10E4 copies/μL, where 10μL is equivalent to 100000 copies) in the positive control gradient dilution solution prepared in the previous step (the total volume after adding water is the same as the sample, and the sample volume depends on the requirements of the reagent kit used). Water can be used as a negative control for preparation.

2.2. Purification of RNA from N+2 samples using a self selected method, this kit is compatible with most viral RNA extraction kits on the market. Recommend using our company's RNA extraction kit for extraction.

3. Set up RT-PCR reaction (30μL system, conducted in the sample preparation room)

3.1. If quantitative analysis is performed and only one repetition is made, label N+9 PCR tubes, of which N+2 are used for the N+2 samples obtained in the previous step, 1 is used for PCR negative control, and 6 are used for the standard curve. If qualitative analysis is performed and only one repetition is made, label N+4 PCR tubes, of which N+2 are used for the N+2 samples obtained in the previous step, 1 is used for PCR negative control, and 1 is used for PCR positive control (using the 4th positive control dilution as a template). The following only describes the steps of quantitative analysis. Qualitative analysis only reduces 6 standard curve reactions to 1, while keeping the rest unchanged.

3.2. Add each component to the labeled tube according to the table below (this table only lists one repetition. The positive control is only set after the sample tube and negative control are set, and the positive control sample is added after all tubes are closed):

Component	Sample tube N+2	RT-PCR negative control tube	RT-PCR positive control (1-6 tubes)
2 × qRT PCR buffer	10μL	10μL	10μL
Novel coronavirus triple dye qRT PCR primer mixture	6μL	6μL	6μL
RNA template obtained from sample preparation (from step 6)	12μL	-	-
Dilute to obtain 6 positive controls (from step 4)	-	-	12 μ L (sample 1 to tube 1, sample 2 to tube 2...)
10 × qRT PCR enzyme mixture	2μL	2μL	2μL

Note: Some qPCR instrument models require the addition of ROX dye calibration to eliminate the optical path difference between tubes and maintain consistency for more accurate experimental data.

Models that require the use of ROX dye I: ABI Prism7000, 7300, 7700, 7900HT, Step-One, Step-One Plus, Suggest a final concentration of 500nM.

Models that require the use of ROX dye II: ABI Prism 7500, 7500Fast, MJ Research's Chromo4 Opticon（II）Corbett Rotor Gene 3000, Suggest a final concentration of 50nM.

3.3. After installation, perform RT-PCR according to the following parameters (parameters may need to be optimized due to different instruments):

Process	Temperature	Time
RT (reverse transcription)	50℃	20 min
Pre denaturation	95℃	10 min
qPCR reaction	95℃	15sec

(40 cycles)	60℃	60 sec (Collect fluorescence signals from SYBR channel)
Perform melting curve analysis according to the preset program of the instrument		

4. **Data processing**

4.1. If this reagent kit is used for quantitative detection, plot a standard curve with the log value of positive control concentration as the horizontal axis and Ct value as the vertical axis. Calculate the log value of the RNA concentration of the sample from the standard curve based on the Ct value of the sample to be tested, and then calculate its concentration.

4.2. If this kit is used for qualitative testing and only determines positive or negative, the negative control Ct must be greater than 35. The positive control must have fluorescence logarithmic growth, typical amplification curve, and Ct value should be less than 33. For the test sample, if its Ct is ≥ 35, it is negative; if it is ≤ 33, it is positive. If it is between 33-35, repeat once. If the Ct value of the repeated experiment is ≥ 35, it is negative; if it is less than 35, it is positive. The melting curve must also be considered. If the melting Tm value differs from the target amplification fragment Tm value by ≥ 2℃, it is non-specific amplification and not true positive amplification.