SARS-CoV-2 qRT-PCR detection assay

(1-plex Taqman™) Manual

【Product】

SARS-CoV-2 qRT-PCR detection assay (1-plex Taqman™)

【Size】 100 reactions/set

【Applications】

The detection assay is only used for 2019 novel Coronavirus (SARS-CoV-2) nucleic acid detection for **research use only (RUO)**, not for diagnoses. The assay should be conducted in a Biosafety level 1 environment with proper safety precautions. For proper biosafety precautions to handle the sample, please refer to the CDC protocol.
https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-for-detection-instructions.pdf

【Principles】

The primers and probes in this assay are designed based on the gene sequences of ORF1ab gene, N gene, RdRP gene and E gene in 2019 novel coronavirus (SARS-CoV-2) genome. The dual-labeled probes can hybridize specifically with a part of the gene sequences. During PCR, the 5' fluorophore from the probe will be digested by Taq polymerase via its 5'-3' exonuclease activity and released from 3' quencher group, and its fluorescence can then be measured quantitatively by qPCR instrument.

【Main components】

Components	Vol (μl)
2×One Step Mix	1000
One step enzyme mix	100
50×ROX Dye 1	40
50×ROX Dye 2	40
Forward Primer (10 μM)	40
Reverse Primer (10 μM)	40
Probe (10 μM)	20
RNase-free water	1000
Positive control plasmid (1 pg/μl)	100

【Storage condition and shelf-life】

Shipping temperature 2~8℃. Store at -20±5℃ away from light, avoid freeze and thaw. Expire 12 months after production date.

【Instrument】

ABI Real-Time PCR Thermal Cyclers with FAM, NED, ROX Channel.

Instrument compatibility:

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qPCR instrument	Dye selection
ABI 7900HT/7300 real time PCR system, and StepOne Plus	Dye 1
ABI 7500, 7500 fast real-time PCR system, Stratagene Mx3000P	Dye 2
Roche, Biorad series of real-time PCR system	No need for dye reference
Other models	Compatibility is unknown. Pilot study recommended

【Sample requirement】

1. Sample: Virus RNA

2. Storage：

<24hr under 2-8℃

< 3 months under -20℃

long-term storage under -70℃ and avoid freeze-thaw

3. Shipping：Sealed foam box with blue ice or dry shipper

【Methods】

1. Reaction mix(in reagent preparation area)

Thaw reagents at room temperature. centrifuge at 1000 rpm for 15 sec, please in a sterile hood and wear gloves, do not touch reaction tube with bare hands.

For each reaction (such as sample, positive control or negative control) , mix the components below:

Components	Vol (µl)/Reaction*			
	RdRP gene	E gene	N gene	ORF1ab
2×One Step Mix	10	10	10	10
One step enzyme mix	1	1	1	1
50×ROX Dye 1/2#	0.4	0.4	0.4	0.4
Forward Primer (10 µM)	0.4	0.4	0.4	0.4
Reverse Prime (10 µM)	0.4	0.4	0.4	0.4
Probe 1 (10 µM)	0.2	0.2	0.2	0.2
Probe 2 (10 µM)	0.2	N.A.	N.A.	N.A.
RNase-free water	Add volume to 19µl			

*Please note the primer and probe quantity are different for different target gene. Please follow the above chart carefully.

1） ABI 7900HT/7300 Real-Time PCR System and StepOne Plus, use 50×ROX Dye 1

2） ABI 7500, 7500 Fast Real-Time PCR System, Stratagene Mx3000P, use 50×ROX Dye 2

2. Sample preparation(In sample preparation area)

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2.1 Nucleic acid extraction

Please refer to the protocol of the corresponding RNA extraction kit.

2.2 Sample loading

Add 1ul (1pg-1000ng) of the testing RNA sample, any of the positive control plasmids depend on the test chosen (ORF1ab gene, N gene, or RdRP gene, or E gene), or negative control (RNase-free water) in the reaction mix from step 1, respectively, to reach a total volume of 20μl/tube. Seal the cap of the tube and centrifuge at 1000rpm for 15 seconds. Transfer the tube to the PCR area.

3. PCR and measurement(PCR area)

Place the PCR tube into real-Time PCR System, and follow the protocol below:

Step	Temperature	Time	Cycle #
Reverse transcript	55℃	15min	1
Pre-denature	95℃	30s	1
Denature	95℃	10s	45
Amplification	60℃	35s (collect information)	

Fluorescence data：Use FAM channel and signal should be collected at 60℃ amplification step.

【Data Analysis】

1. Quality control:

1. For Negative Control： no typical S-shaped curve for the targeted gene, Ct>40
2. For Positive Control：S-shaped curve with Ct ≤30。
3. Failure to obtain either above indicates the failure of the test, and the sample should be re-tested or test in an alternative method.

2. Interpreting Test Results：

Data can be interpreted as follows if the quality control standard mentioned above is met.

1. Positive：Sample Ct in both fluorescent channels should be positive before or at 37 cycles, with an S-shaped curve including log-phase.
2. When to repeat the assay: If one of the fluorescent channels have Ct value ≤37, while the other channel Ct value between 37-40, we suggest repeating the test to confirm. If the repeated test still shows 37<Ct≤40, and the S-shaped curve shows the log phase, the result can be considered as positive.
3. Netagive: All fluorescent channels have Ct＞40, or no signal, the result is considered negative.

【Assay limitations】

- This assay is for **Research Use Only (RUO)** and not for diagnostic purposes. We make no claims on the performance of this assay.
- This assay's design is impacted by the accuracy of the publically SARS-CoV-2 genome sequence.
- This assay's performance is impacted by a range of uncontrolled and un-tested factors such as sample quality, various sample extraction methods, sample cross-contamination, and data analysis variation.
- This assay may have cross-reactivity with other coronavirus family members such as causative agents of the Middle East Respiratory Syndrome (MERS) or Severe Acute Respiratory Syndrome (SARS).

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- Stability tests and data are not available at this moment due to the emergency and time limits. We can not guarantee the accuracy of the shelf life, storage conditions, efficiency, etc. We will update the information once we have more data.

【Biosafety precautions】

1. Analysis performs this assay requires proper prior training and is familiar with the testing procedures and interpretation of the results.
2. To ensure the quality and accuracy of the assay, use new DNase and RNase free disposable tubes and pipettes tips, calibrate pipettes or liquid handler before use.
3. Separate the area for different process of the assay
    a. Reagent preparation area：prepare all reagents and reaction mix within this area
    b. Sample preparation area: prepare the testing sample, positive and negative samples
    c. PCR area：To void contamination, do not use instruments and consumables other than its intended purpose. Clean the bench after each experiment.
4. Thaw the reagents in room temperature completely, mix well and centrifuge shortly before use.
5. Sample preparation should be performed in a certified class II biological safety cabinet following biosafety level 2 or higher.
6. Each round of experiments need to have negative and positive control. Do not mix and match reagents with different lot#. Do not use reagents that are expired.
7. For nucleic acid sample stored at -70℃, thaw it in room temperature completely, mix well and centrifuge shortly before use.
8. Seal the reaction mix tube/plate, or close the tube/plate cap completely when transferring to the sample preparation area.
9. Submerge the pipette tip completely in the reaction mix when adding samples. Do not leave any trace of the sample on the side of the tube or plate. Close the cap immediately after adding the samples.
10. Avoid bubbles when aliquoting the reaction mix. Check if the cap is completely sealed before load it on the PCR thermal cycler to avoid contamination of the instrument.
11. Calibrate Real-Time PCR Thermal Cyclers, and clean its sample plate stage after each use.
12. Decontaminate work surfaces and equipment with appropriate disinfectants follow manufacturer's recommendations.
13. The samples used this test are considered as infectious biohazardous material. After samples are processed, dispose all samples and samples containing tubes/plates, PCR tubes/plates and pipette tips following "Biosafety in Microbiological and Biomedical Laboratories (BMBL) – Fifth Edition" by CDC or your country's biosafety regulation.

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