DirectDetect™ SARS-CoV-2 Detection Kit (PCR-Fluorescence Probe) User Manual

For in vitro diagnostic use

For Emergency Use Authorization Only

【Device Name】

DirectDetect™ SARS-CoV-2 Detection Kit (PCR-Fluorescence Probe)

【Specification】

24 tests/kit; 48 tests/kit; 96 tests/kit.

KitA Compatible ABI7500,BIO-RAD CFX96

【Intended Use】

The DirectDetect™ SARS-CoV-2 Detection Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in nasopharyngeal/oropharyngeal swabs, sputa from individuals meeting CDC coronavirus disease 2019 clinical criteria (e.g., clinical signs and symptoms associated with COVID-19) in conjunction with CDC SARS-CoV-2 epidemiological criteria (e.g., history of residence in or travel to a geographic region with active SARS-CoV-2 transmission at the time of travel, or other epidemiologic criteria for which SARS-CoV-2 testing may be indicated).

This kit targets the ORF1ab and N gene of SARS-CoV-2, which offers an auxiliary means for the diagnosis of the SARS-CoV-2 infected patients, and is used for epidemiological surveillance. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal/oropharyngeal swabs, or sputa during the acute phase of infection. Positive results are presumptive identification of active infection but do not rule out co-infection with bacteria or viruses. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Because of the characteristic ingredients and unique reaction programs of the kit, which can release the target nucleotide from specimens and eliminate the inhibitor, nucleic acid extraction is unnecessary when using this kit. The outcome of the kit is an accurate, sensitive, efficient and stable detection result.

Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

【Test Principle】

This kit adopts the rRT-PCR method combined with fluorescence probes to detect the conserved region of ORF1ab and N gene of SARS-CoV-2, covered by a pair of specific primers and a fluorescence-labeled probe, respectively.

The probe for ORF 1ab gene of SARS-CoV-2 detection is labeled by FAM detector and BHQ quencher at the terminal ends of oligonucleotides. ROX is the detector for the N gene. If SARS-CoV-2 is present in the sample, the target sequence will be amplified and the fluorescence-labeled probe will be degraded by exonuclease activity of Taq DNA polymerase, resulting in a concomitant increase in fluorescent signal.

In addition, an internal control is set with a HEX detector labeled probe and a pair of primers using the RNase P gene of human as the target, to monitor the amplification effect of the detection system.

As the kit can release the target nucleotide from specimens and tolerate the inhibitor, nucleic acid extraction is unnecessary when using this kit. From the RNA release to the end detection result, the entire process is achieved in a single tube, which can reduce the possibility of contamination and the biosafety level of detection. The end result, which occurs after one hour of test kit duration, should be accurate, sensitive, and efficient.

【Kit Contents】

Table 1. The Contents of the Test Kit A（Compatible ABI7500,BIO-RAD CFX96 et.）

Contents	Components	Specification/Quantity		
		24 tests/kit（6018001902-EN）	48 tests/kit（6018001906-EN）	96 tests/kit（6018001908-EN）
SARS-CoV-2 PCR Mix I	Reverse transcriptase, Taq DNA polymerase, dNTPs,$MgCl_2$ and other buffers	840μL/vial ×1	840μL/vial ×2	840μL/vial ×4
SARS-CoV-2 PCR Mix II	Primers and probes for ORF1ab and N gene of SARS-CoV-2, and RNaseP gene of human detection	80μL/vial ×1	160μL/vial ×1	320μL/vial ×1
SARS-CoV-2 Positive Control	Pseudovirus with the ORF1ab and N gene of SARA-CoV-2	120μL/vial ×1	240μL/vial ×1	480μL/vial ×1
Negative Control	Sample storage solution and RNP Pseudovirus	120μL/vial ×1	240μL/vial ×1	480μL/vial ×1
Respiratory sample buffer	Base Lye solution	500μL/vial ×1	500μL/vial ×2	500μL/vial ×4

Table 2. Product Accessories

Contents	Components	Intended use	Specification/Quantity		
			24 tests/kit	48 tests/kit	96 tests/kit
Sample storage solution	Hank's solution and RNA stabilizer	Nasopharyngeal/oropharyngeal swabs Specimen Storage	500μL/vial ×24	500μL/vial×48	500μL/vial×96
Sputum liquification buffer	DTT	Liquefaction treatment for Sputum	50μL/vial×1	100μL/vial×1	200μL/vial×1
		Specimen			

Note: 1.Different lots batches of components in the kit are not interchangeable.

2.Product Accessories need to be purchased separately.

【Storage and Period of Validity】

All contents should be stored at -20±5 and avoid direct light.

Repeated thawing and freezing (>4x) should be avoided.

Opened reagents in vials can be stored 48 hours at 2-8 and should be used within 5 days.

The validity period is tentatively 6 months, and the kit can be used until the indicated expiration date on the kit label.

Production date and period of validity are shown on the kit label.

【Specimen Type Requirements】

1. Specimen Type: nasopharyngeal/oropharyngeal swabs and sputum.
2. Specimen Collection Objects: suspect individuals of patients with COVID-19, suspected cluster of cases, and others suspected of being infected with SARS-CoV-2.
3. Swab Sampling Requirements

Sampling swab type: Use only synthetic fiber swabs with plastic (aluminum) shafts. Do not use cotton swabs or swabs with wooden shafts.

4. Specimen Collection

4.1 Nasopharyngeal swab: Insert a swab into the nostril parallel to the palate, and leave the swab in place for a few seconds to absorb secretions. Place swab immediately into a sterile tube containing sample storage solution.

4.2 Oropharyngeal swab: Swab the bilateral pharyngeal tonsils and posterior pharynx using the swab. Place swab immediately into a sterile tube containing sample storage solution.

4.3 Sputum: Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.

5. Specimen Storage

Fresh samples from patients can be tested immediately. The fresh samples can also be stored at 2-8°C for 4 days, or at -20±5°C for 3 months. Long-term preservation (>3 months) should be placed at a temperature below -70°C. Thawing and freezing repeatedly (>4X) is not recommended.

【Applicable Instrument】

ABI 7500 Real-time PCR System, BIO-RAD CFX96 Real-time PCR System

【Procedure】

1. Amplification Reagents Preparation

1.1 Take out the Contents of SARS-CoV-2 PCR Reaction Mix I, SARS-CoV-2 PCR Reaction Mix II, SARS-CoV-2 Positive Control, Negative Control and Respiratory sample buffer, Sputum Liquefaction buffer(only use for Sputum Specimen). To dissolve completely, mix all contents separately by vortexing for 10 seconds, then short-spin for 10 seconds.

1.2 (N+2) test PCR Mix should be prepared as follows when N specimens are being tested:

(1)Pipette (N+2) × 32 μL of SARS-CoV-2 PCR Reaction Mix I and (N+2) × 3 μL of SARS-CoV-2 PCR Reaction Mix II into a new sterile PCR tube.

(2) Mix by vortexing for 10 seconds and short-spin for 10 seconds.

(3) Dispense the prepared Amplification Reagent into new sterile PCR tubes and close the caps.

Table4. Amplification Reagent Preparation

Contents	Kit A
	ABI7500/CFX96
SARS-CoV-2 PCR Reaction Mix I	32μL
SARS-CoV-2 PCR Reaction Mix II	3μL
Total volume of Amplification Reagent	35μL

2. Specimen Treatment and Adding

2.1 Nasopharyngeal /oropharyngeal swab: Pipette 15μL of the swab sample into a new sterilized tube and add 15μL of the Respiratory sample buffer. Mix completely. Pipette 15μL of the mixture into the Amplification Reagent.

2.2 Sputum sample: Dilute the Sputum liquefaction buffer by 50-fold with 0.01M of phosphate buffered solution with pH 7.2. Add the diluted sputum liquefaction buffer to an equal volume of specimen. Incubate the mixture at 56°C with intermittent mixing until the sample is liquefied (up to 30 minutes). Pipette 2μL of liquefied sample into the Amplification Reagent to test.

2.3 Control:

Pipette 15μL of one positive control into the Amplification Reagent and pipette 15μL of one negative control into the Amplification Reagent.

3. PCR Amplification

3.1 Put the PCR tube into the PCR instrument and record the sample order.

3.2 Thermal protocol setup is as follows:

Table 5. Parameter Instrument Settings for ABI7500 and BIO-RAD CFX96

Pro.	Temp.	Time	No. of Cycles
1	42	5 minutes	1 cycle
2	95	10 seconds	15 cycles
	50	15 seconds	
3	95	1 minute	1 cycle
4	95	10 seconds	30 cycles
	55	30 seconds (acquire fluorescence)	

3.3 Channel definition

(1) Select the FAM channel to test the ORF 1ab gene, select the ROX channel to test the N gene, and select the VIC/HEX channel to test the Internal Control.

(2) The Passive References should be set as none on the ABI7500 instrument.

3.4 Quality Control

(1) Positive and Negative Control

Positive Control: The detection curves of FAM, ROX channels should have the logarithmic growth period with the Ct value≤ 25.

Negative Control: The detection curves of FAM and ROX channels should be none detected. The VIC/HEX channel should have the logarithmic growth period with the Ct value≤ 25.

If the detection results for the positive and negative controls are consistent with the results above, the test is credible. Otherwise, re-test again.

(2) Internal Control

Internal controls can monitor the effect of nucleic acid release from the pathogen tested and the inhibitor of specimens. For the negative specimens, the internal controls should be positive. For the positive specimens, the internal controls can be positive or negative. Otherwise, the test is not credible, and should be re-tested again.

【Cut-Off】

The cut-off of Ct value is 29.00, according to the ROC curve for a large number of clinical positive specimens and negative specimens. So, if the value of Ct≤ 29.00 for a specimen, the detection result is positive; otherwise, the result is negative.

【Interpretation of Results】

According to the parameters of FAM, ROX and VIC/HEX channels, after thresholds and baselines are adjusted according to instrument manuals, obtain the detection result of every channel.

1. Quality Control Analysis

The positive control, negative control and internal control should all meet the above quality controls.

2. Results Analysis

For FAM, ROX, or VIC/HEX channels, if the values of Ct≤ 29.00, the detection result is positive. Otherwise, the detection result is negative.

Table 6. Interpretation of Results

No.	FAM	ROX	HEX/ VIC	Target Detection	Result Interpretation
1	+	+	+/-	ORF1ab Positive； N gene Positive	Presumptive positive SARS-CoV-2
2	+	-	+/-	ORF1ab Positive； N gene Negative	Repeat the testing. If repeat result is positive, it is presumptive positive SARS-CoV-2.
3	-	+	+/-	ORF1ab Negative； N gene Positive	Repeat the testing. If repeat result is positive, it is presumptive positive SARS-CoV-2.
4	-	-	+	ORF1ab Negative； N gene Negative	SARS-CoV-2 not detected
5	-	-	-	Invalid	Repeat testing. If the repeated result remains invalid, consider collecting a new specimen from the patient.

Note: "+" represents a positive result; "-" represents a negative result.

*The internal control can be positive or negative for cell cultures detection. The internal control should be positive for negative clinical specimen. Otherwise, the test is not credible and a new sample should be collected and tested.

【Limitations of the Assay】

1. This kit's detection results should not be solely used to diagnose a patient. Instead, the results should be considered in addition to clinical symptoms and medical examination for diagnosis. The positive results are considered presumptive until confirmed.
2. False negative results may be caused by suboptimal conditions during specimen collection, transport and storage, as well as a virus titer lower than the LOD of this kit. Specimen type variation and time of sampling time also may also cause false negative results due to limited recognition of the new virus. So, sampling a variety of specimen types at multiple times in a single patient may reduce the likelihood of obtaining a false negative result.
3. False positive results also may be caused by contamination of the amplification product and cross-contamination of specimens during collection, transport, and storage.
4. The results may vary with different specimen sample types and/or different time points.

【Product Performance】

1. The limit of detection (LoD) is 3.75 copies/rxn, for both the ORF 1ab gene and N gene.
2. There is no cross reaction by human DNA or other pathogens as follows:

Table 7. Pathogens for Cross Reactivity

FluB/Victoria	FluB/Yamataga	Seasonal H1N1 influenza virus
H5N1	H7N9	New influenza A H1N1 virus (2009)
H3N2	Mycoplasma pneumoniae	Respiratory syncytial virus type A
Adenovirus 3	Adenovirus 7	Respiratory syncytial virus type B
Chlamydia pneumoniae	Chlamydia pneumoniae	Cytomegalovirus
CA16	Coronavirus 229E	Coronavirus OC43
EV71	Coronavirus HKU1	Coronavirus NL63
HBoV-1	Parainfluenza virus PIV2	Partial pulmonary virus type A
Rhinovirus	E. coli	Legionella pneumophila
Streptococcus pneumoniae	C. albicans	Mycobacterium tuberculosis attenuated strain
Klebsiella pneumoniae	Haemophilus influenzae	Staphylococcus aureus

【Warnings and Precautions】

1. For In Vitro Diagnostic (IVD) use only. Carefully read the instructions prior to starting.
2. CoyoteBio Sample storage solution is recommended as it can increase pathogen concentration by unit volume, may possibly leading to a positive detection result despite a low pathogen concentration.
3. Usage of the product beyond validity date and different batches of components is prohibited.
4. After dissolving completely, vortex and short-spin before using kit reagents.
5. Use RNase- & DNase-free tubes and pipette tips with this kit.
6. When adding a sample to the PCR Mix, avoid getting any sample onto the wall of PCR tube and close the tube immediately after.
7. Clinical specimens should be treated as potentially infectious material, and should be operated in a biosafety cabinet.
8. Clinical laboratories should be certified with proper bio-safety containment equipment and molecular biology qualifications.
9. The type described as above 【Specimen type Requirements】 was for verification only; the detection performance is not optimal for other specimen types and methods.

【Sign Interpretation】

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【References】

[1] Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected. WHO.2020.

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[3] Zou L, Ruan F, Huang M, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients[J]. New England Journal of Medicine, 2020.

[4] Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study[J]. The Lancet Respiratory Medicine, 2020.