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

[Device Name]

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

[Specification]

Specification A:24 tests/kit; 48 tests/kit; 96 tests/kit.

[Intended Use]

The DirectDetect™ SARS-CoV-2 Detection Kit is a real-time RT-PCR test intended for the qualitative detection of ORF1ab and N gene of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in oropharyngeal swabs and sputa specimen from individuals that are suspected Corona Virus Disease 2019 cases, suspected clustered cases, or that needs to be infectious diagnosis or differential diagnosis.

Detection of nucleic acid of novel coronavirus should meet the requirement of *Technical Guidelines for Covid-19 Laboratory Testing* and should ensure the biosecurity.

The test result of kit is only for clinical reference, and should not be used as sole criteria for clinical diagnosis. It is recommended to combine the patient's clinical manifestations and other laboratory test result for a comprehensive analysis of the patient's condition.

The kit is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of PCR amplification and molecular biological testing. The kit is intended for use in laboratory withqualified biology security facility and protection procedures.

[Test Principle]

This kit adopts the PCR method combined with fluorescence probes to detect the conserved region of ORF1ab and N gene of SARS-CoV-2. The specific primer and probe is designed for detection of RNA of SARS-CoV-2.

The probe for detection of ORF1ab and N gene of SARS-CoV-2 in the kit is oligonucleotides labeled with FAM or ROX reporter and BHQ quencher at the terminal ends. The fluorescence emitted by the reporter is absorbed by the quencher when the probe is intact. 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 separation of reporter and quencher and a concomitant increase in fluorescent signal monitored by fluorescence detection system. In addition, the kit has an Internal Control (IC) system, internal reference RNase P primer and probe to monitor sampling and identify possible RNA transcription and PCR amplification inhibition.

[Kit Contents]

Table1 Contents of the Kit Specification A

Contents	Key Components	Specification (24 tests/kit)	Specification (48 tests/kit)	Specification (96 tests/kit)
SARS-CoV-2 PCR Mix I	Reverse transcriptase, Taq DNA polymerase, Tris buffer, Enhancer, dNTPs, $MgCl_2$ and others	840μL/vial×1 vial	840μL/vial×2 vials	840μL/vial×4 vials
SARS-CoV-2 PCR Mix II	Specific primers and probes for ORF1ab, N gene of SARS-CoV-2, and human RNaseP gene	80μL/vial ×1 vial	160μL/vial×1 vial	320μL/vial×1 vial
SARS-CoV-2 Positive Control	Contains non-infectious armored RNA of SARA-CoV-2	120μL/vial×1 vial	240μL/vial×1 vial	480μL/vial×1 vial
Negative Control	Contains non-infectious armored RNA with RNase P sequence in sample storage solution	120μL/vial×1 vial	240μL/vial×1 vial	480μL/vial×1 vial
Respiratory Sample Buffer	Base solution	500μL/vial×1 vial	500μL/vial×2 vials	500μL/vial×4 vials

Note: 1. Specification A is usedin ABI7500 and BIO-RAD CFX96.

2. Different batches of components in the kit are not interchangeable.

Other Components Required but Not Included with the Kit:

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

(2) Swab sample storage buffer and tube：VTM or UTM, e.g.Coyote sample storage buffer (JS WX FDA Filing 20190148) and Yocon MT0301 Viral Transport Medium (BJ FDA (A) 20182400236).Please do not use a preservation solution with inactivation or lytic virus action.

[Storage and Period of Validity]

Stored at -20±5℃ and avoid direct light. The validity period is 12 months.

Repeated thawing and freezing (>4x) should be avoided. Opened reagents in vials can be stored 4 days at 2-8℃.

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

[Specimen Type Requirements]

1. Specimen Type: Oropharyngeal swabs.

2. Specimen Collection Objects:

Individuals that are suspected Corona Virus Disease 2019 cases, suspected clustered cases, or that needs to be infectious diagnosis or differential diagnosis.

3. Requirements of swab sampling, storage buffer and tube

3.1 Sampling swab type: Use only swabs with a synthetic tip (such as polypropylene fiber) or flocking tip with plastic shafts. DO NOT use cotton swabs or swabs with wooden shafts.

3.2 Swab sample storage buffer and tube: Coyote sample storage buffer (JS WX FDA Filing 20190148) and Yocon MT0301 Viral Transport Medium (BJ FDA (A) 20182400236) for oropharyngeal swabs.

4. Specimen Collection

4.1 Oropharyngeal swab: Swab the bilateral pharyngeal tonsils and posterior pharynx using the swabs with polypropylene fiber tip or flocking tip with plastic shafts. Place swab immediately into a sterile tube containing sample storage solution, and discard the tail. Close the storage tube.

5. Specimen Storage

Fresh samples from patients should be tested immediately. The fresh samples can also be stored at 2-8°C for 5 days. 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

Take out the Contents of SARS-CoV-2 PCR Mix I, SARS-CoV-2 PCR Mix II, SARS-CoV-2 Positive Control, Negative Control and Respiratory Sample Buffer. Dissolve reagent completely, mix all contents separately by vortexing, then short-spin for 5-10 seconds. (N+2) test PCR Mix should be prepared as follows when N specimens are being tested: Pipette (N+2) × 32 μL of SARS-CoV-2 PCR Mix I and (N+2) × 3 μL of SARS-CoV-2 PCR Mix IIinto a new sterile PCR tube. Mix by vortexing. Dispense 35μL of the prepared Amplification Reagent into new sterile PCR tubes and close the caps. The remaining reagent store at -20±5℃.

Table 2 Amplification Reagent Preparation

Contents	SARS-CoV-2 PCR Mix I	SARS-CoV-2 PCR Mix II	Total
Volume	32μL	3μL	35μL

2. Specimen Treatment

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.

The positive control and negative control should be treated same as the swab specimen.

Note: Avoid to pipette the viscous part of specimens.

3. Add Sample

Pipette 15μL of treated specimen (treated oropharyngeal swab sample or liquified and diluted sputum sample), treated negative control, and treated SARS-CoV-2 positive control into the tube containing amplification reagent. Mix completely and then short-spin.

4. PCR Amplification

4.1 Put the PCR tube into the PCR instrument and record the sample order. The PCR instrument setup is as follows:

The parameter in the following table is for ABI7500 and BIO-RAD CFX96.

Table 3Instrument Parameter Settings

Pro.		Temp.	Time	No. of Cycles
1	Transcription	42℃	5 minutes	1 cycle
2	Pre-amplification	95℃	10 seconds	15 cycles
		50℃	15 seconds	
3	Pre-denature	95℃	1 minute	1 cycle
4	Amplification and fluorescence collection	95℃	10 seconds	30 cycles
		55℃	30 seconds* (acquire fluorescence)	

Passive Reference should be set as none in ABI 7500.

4.2 Select FAM channel to test the ORF1ab, select the ROX channel to test the N gene, and select the VIC/HEX channel to test the Internal Control.

4.3 Quality Control

Negative Control: The Ct value in FAM and ROX channels should beUndetermined (ABI 7500), N/A or NaN (CFX96), without significant amplification curve. The VIC/HEX channel should have significant exponential amplification curve with the Ct value≤20.00.

Positive Control: The detection curves of FAM, ROX channels should have the significant exponential amplification curve with the Ct value≤20.00.

The test is valid when the positive control, negative control and internal standard results all meet the preset values.

[Cut-Off]

The Cut-off of Ct value is determined by ROC-curve.

Fluorescence Channel	Target gene	Cut-off	Interpretation of the result
FAM	ORF1ab	27	If the Ct value of testing sample is≤27.00, the result is positive. Otherwise, it is negative.
ROX	N	27	If the Ct value of testing sample is≤27.00, the result is positive. Otherwise, it is negative.
HEX (or VIC)	RNP IC gene	27	If the Ct value of testing sample is≤27.00, the result is positive. Otherwise, it 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.

All test controls should be examined and meet the preset values prior to interpretation of patient results.

For FAM, ROX, or VIC/HEX channels, if the Ct values ≤ cut-off and have the significant exponential amplification curve, the detection result is positive. If Ct value＞cut-off or

Undetermined (ABI 7500), N/A or NaN (CFX96) is shown, the detection result is negative.

Table4 Interpretation of Results

No.	FAM Channel	ROX Channel	HEX/VIC Channel	Determination of detection	Result Interpretation
1	+	+	+/-	ORF1ab Positive; N gene Positive	Presumptivepositive SARS-CoV-2.
2	+	-	+/-	ORF1ab Positive; N gene Negative; Repeat the testing after re-sampling	If repeat result is positive for ORF1ab or N gene, it is presumptive positive SARS-CoV-2. Or presumptive SARS-CoV-2 negative.
3	-	+	+/-	ORF1ab Negative; N gene Positive Repeat the testing after re-sampling	If repeat result is positive for ORF1ab or N gene, it is presumptive positive SARS-CoV-2; or presumptive SARS-CoV-2 negative.
4	-	-	+	ORF1ab Negative; N gene Negative	Presumptive SARS-CoV-2 negative.
5	-	-	-	Invalid. Recommend to re-collect and test the sample.	/

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

*The internal control can be positive or negative ("+/-") for cell cultures detection. The internal control should be positive for SARS-CoV-2 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 combination with clinical symptoms and medical examination for diagnosis.

2. Analysis of possibility of false negative results

2.1 Improper process of specimen collection, transport and storage and low virus titer in the sample may cause the false negative results.

2.2 Sequence variation occurs in the SARS-CoV-2 may have the risk of false negative results.

2.3 As sudden occurrence of SARS-CoV-2, the optimal sample type and sampling time are not thoroughly validated. Sampling a variety of specimen types and sampling at multiple times for the same patient may reduce the likelihood of false negative result.

3. Analysis of possibility of false positive results

Improper process of specimen collection, transport and storage, contamination of laboratory and reagent, and cross-contamination of specimens may cause the false positive results.

4. The results may vary with different specimen sample types and/or different time points.

[Product Performance]

1. Positive Coincidence Rate: The kit is used to test positive standards P1-P7 in Chinese national standard of SARS-CoV-2 nucleic acid detection reagent, and the positive coincidence rate is 100%. The kit is used to test positive references P1-P14 in enterprise reference. The test result is ORF1ab positive and N gene positive for P1-P14, and the positive coincidence rate is 100%.

2. Negative Coincidence Rate: The kit is used to test negative standards N1-N22 in national standard of SARS-CoV-2 nucleic acid detection reagent, and the negative coincidence rate is 100%. The kit is used to test negative references N1-N14 in enterprise reference. The test result is ORF1ab negative and N gene negative for N1-N14, and the positive coincidence rate is 100%.

3. Precision for testing standards:

The precision standard in Chinese national standard of SARS-CoV-2 nucleic acid detection reagent was diluted by 20-fold and tested by 10 times repeated in ABI7500 and Flash20. The testing result is positive and the coefficient of variance (CV, %) of Ct value for each channel is no more than 5.0%.

4. The Limit of Detection: The limit of detection (LoD) for the kit is 4×10^2 copies/mL.

The testing result of sensitivity standard S1-S3 in Chinese national standard of SARS-CoV-2 nucleic acid detection reagent is positive.

5. Specificity:

Pathogens causing similar symptoms or likely in the circulating area with SARS-CoV-2 virus and human DNA were tested with the kit. None of the pathogen or human DNA showed any cross-reactivity with the kit. The tested pathogens include Coronavirus HKU1/NL63/229E/OC43/SARS/MERS, Influenza A virus/2009 H1N1, Influenza A virus/sH1N1, H3N2, H5N1, H7N9, Influenza B virus/Yamagata, Influenza B virus/Victoria, Human respiratory syncytial virus A, B, Parainfluenza type 1, 2, 3, Rhinovirus group A, B, C, Adenovirus type 1, 2, 3, 5, 7, Enterovirus group A, B, C, D, Human pneumovirus/metapneumovirus, Measles virus, Human cytomegalo virus, Rotavirus, Norovirus, Mumps virus, Human herpesvirus, Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella pneumonia, Bordetella pertussis, Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Klebsiella pneumoniae, Mycobacterium tuberculosis, Aspergillus fumigatus, Candida albicans, Candida glabrata, Cryptococcus neoformans.

6. Clinical evaluation:

833 specimens were tested in three clinical sites and were head-to-head compared with commercialized product. The positive coincidence rate is 98.27%, negative coincidence rate is 97.91%, and the total coincidence rate is 98.07%.

[Warnings and Precautions]

1. For in vitro diagnosis use only. Carefully read the user manual before use.
2. Usage of the product beyond validity date and different batches of components is prohibited.
3. Dissolve completely, vortex and short-spin before using kit reagents.
4. Use RNase- & DNase-free tubes and pipette tips with this kit.
5. When adding a sample to the PCR Mix, the sample should be mixed with amplification reagent and avoid getting any sample onto the wall of PCR tube.
6. The specimen type, collection and processing methods described as above [Specimen Type Requirements] was validated with the kit. The performance may not be up to for other sample types.

[Mark Interpretation]

IVD	In Vitro Diagnosis Products
CE	CE marking
REF	Reference
LOT	Lot number
	Manufacturing date
	Use by
	Manufacturer
-15℃ -25℃	Temperature limitation(-15℃~-25℃)
EC REP	European Authorized Representative

【Company Information】

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