ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit

【Product Name】

ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit

【Package Specifications】

100 tests/kit

【Catalogue Number】

3103010069

【Intended Use】

ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit is a real-time reverse transcription polymerase chain reaction (RT-qPCR) test intended for qualitative detection of nucleic acid from the SARS-CoV-2 in oropharyngeal swabs.

Results are the detection of SARS-CoV-2 RNA. The RNA is generally detective in oropharyngeal swab during the acute phase. Positive results are indicative of the presence of SARS-CoV-2 RNA. Positive results do not rule out bacterial infection or co-infection with other viruses.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, recent exposures and epidemiological information.

The level of SARS-CoV-2 that would be present in oropharyngeal swabs from individuals with early infection is unknown. Therefore, negative results must be combined with clinical observations, patient history, and epidemiological information.

【Principle of the Procedures】

The ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit is a one-step real-time reverse transcription polymerase chain reaction (RT-qPCR) test for qualitative detection of SARS-CoV-2 specific RNA.

The ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit includes all reagents needed for RT-qPCR, 3 sets of primers and probes designed to detect the SARS-CoV-2 RNA in oropharyngeal swabs and 1 set of primers and probes designed to detect the RNase P. The RNase P serves as cellularity control for sample collection, nucleic acid extraction and PCR amplification.

The ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit is a one-step RT-qPCR test in a single tube that first reverse transcribes specific RNA templates into cDNA and then subsequently amplified by QuantStudio™ 5 Real-Time PCR System or Bio-Rad CFX-96 Deep Well Dx Systems. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by Real-Time PCR System.

【Components of the Diagnostic Kit】

Component	Specification	Quantity	Description
RT-qPCR Reaction Mix	850μL/tube	1	Composed of reagent for amplification and probes and primers of target genes and internal reference
Enzyme Mix	150μL/tube	1	Taq polymerase, Reverse transcriptase and UDG
Positive Control	1000μL/tube	1	Mix solution of pseudo-viruses with target virus genes and internal reference
Negative Control	1000μL/tube	1	DNase/RNase free water

【Materials Required but not Provided】

- ANDiS 350 Automated Nucleic Acids Extraction System (Cat.3105020003)
- ANDiS Viral RNA Auto Extraction & Purification Kit (Cat. 3103010025,64 Tests)
- Microcentrifuge
- Vortex mixer
- Single- and multi-channel pipettes
- Pipette tips (10 μL, 200 μL and 1000 μL tips with filters are preferred)
- 100% ethanol, ACS reagent grade or equivalent
- 1.5 mL microcentrifuge tubes (DNase/RNase free)
- 2 mL microcentrifuge tubes (DNase/RNase free)
- 0.2 mL PCR reaction plates/ tubes
- MicroAmp Optical 8-tube Strips (Applied Biosystems; catalog #4316567).
- MicroAmp Optical 8-cap Strips (Applied Biosystems; catalog #4323032)

【Storage and Shelf-life】

- The kit is stored at -20±5℃, and the shelf-life is 12 months.
- Protect RT-qPCR Reaction Mix which contains fluorogenic probes from light, and keep reagent and reaction tubes capped or covered as much as possible.
- Enzyme Mix must be thawed and kept on a cold block at all times during preparation and use. Avoid more than 10 cycles of freeze-thaw.
- Always check the expiration date prior to use. Do not use expired reagents.

【Applicable Instruments】

- ABI QuantStudio™ 5 Real-Time PCR Instrument (96 well, 0.2mL Block)
- Bio-Rad CFX-96 Deep Well Dx Systems

【Specimen Requirements】

1. Applicable specimen type: oropharyngeal swab

2. Sample collection：

Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients. Follow specimen collection devices manufacturer instructions for proper collection methods.

Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron®, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing viral media.

3. Sample storage and transportation

For oropharyngeal swab specimens store specimens at 2-8℃ and ship overnight to testing facility on ice pack. If a specimen is frozen at -70℃ or lower, ship overnight to testing facility on dry ice. Specimen store at 2-8℃, it is recommended not to exceed 1 day. Specimen store at -20±5℃, it is recommended not to exceed 7 days. Repeated freezing and thawing of samples should be avoided.

Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens.

【Warnings and Precautions】

- The components in the kits of different batches cannot be interchanged.
- This product is used for in vitro test only. Please read the Instruction carefully before experiment.
- All user, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the test independently.
- Handle all specimens as if infectious using safe laboratory procedures, Specimen processing should be performed in accordance with national biological safety regulation.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).
- If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
- Use separate and segregated working area for (1) specimen preparation, (2) reaction set-up and (3) amplification/detection activities. Workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering different areas.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Change aerosol barrier pipette tips between all manual liquid transfers.
- Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves between samples and whenever contamination is suspected
- RNA should be maintained on cold block or on ice during preparation and use to ensure stability.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.
- Different batch of products should not be conducted on cross-utilization.

【Test Method】

1. RNA Extraction

Specimens, positive control and negative control should be extracted before PCR amplification.

RNA should be extracted using Nucleic Acid extracting Kit in line with the manufacturer's instructions. The assay was validated by the recommended RNA extraction kits (Cat. 3103010024, 16 Tests; Cat. 3103010025, 64 Tests; Cat. 3103010026, 128 Tests) by 3D Biomedicine Science & Technology Co., Limited.

200μL of each specimen, Positive Control and Negative Control are needed to extract nucleic acid automatically using ANDiS 350 Automated Nucleic Acids Extraction System（Cat.3105020003）by 3D Biomedicine Science & Technology Co., Limited.

2. Reagent preparation

Equilibrate all the reagents and controls except Enzyme Mix to room temperature. Thaw the Enzyme Mix in cooler or on ice. Mix all the reagents and controls by vortex for 10 seconds, centrifuge briefly to collect the content to the bottom of the tube. Preparation of RT-qPCR Mix according to the formula described in the Table below.

Reagent Name	Volume in μL per Reaction	Volume in μL per N Reactions
RT-qPCR Reaction Mix	8.5	8.5 × (N+1)
Enzyme Mix	1.5	1.5 × (N+1)
Total Volume	10	10 × (N+1)

Add 10μL of RT-qPCR Mix into each required well of an appropriate optical 96-well reaction plate or optical 8-tube strip.

3. Add sample

Add 10μL of extracted RNA of samples, Positive Control and Negative Control into each well or tube containing 10μL of RT-qPCR Mix. Seal the optical 96-well reaction plate with optical adhesive file or cap the optical 8-tube strip with optical cap. Spin the optical 96-well reaction plate or optical 8-tube strip briefly to collect the content to the bottom of the tube.

4. PCR amplification

4.1 Setting of QuantStudio™ 5 Real-Time PCR System

4.1.1 Define the general setting:

Reference Dye: None

Sample Volume: 20μL

Note: "None" should be selected in the "Select the dye to use for passive reference" since the default is "ROX".

4.1.2 Define the Fluorescent Detectors (Dye)

Detection	Reporter Dye	Quencher
SARS-CoV-2 specific RNA (ORF1ab)	FAM	None
SARS-CoV-2 specific RNA (N gene)	ROX	None
SARS-CoV-2 specific RNA (E gene)	VIC	None
Internal Control	CY5	None

4.1.3 Set up RT-qPCR Thermal Cycle profile:

"Experiment Properties" - "Run mode" choose "Fast".

Stage	Temperature	Time	Cycle number	Ramping Speed
1	50℃	2 minutes	1	3.19℃/s
2	95℃	2 seconds	1	3.19℃/s
3	95℃	1 second	41	3.19℃/s
	60℃	13 seconds		2.45℃/s

Note: Collect fluorescent signal at 60℃ step.

4.2 Setting of Bio-Rad CFX-96 Deep Well Dx Systems

4.2.1 Set up RT-qPCR Thermal Cycle protocol

Stage	Temperature	Time	Cycle number
1	50℃	2 minutes	1
2	95℃	2 seconds	1
3	95℃	1 second	41
	60℃	13 seconds	

4.2.2 Select Fluorophores (Dye)

Detection	Reporter Dye	Quencher
SARS-CoV-2 specific RNA (ORF1ab)	FAM	None
SARS-CoV-2 specific RNA (N gene)	ROX	None
SARS-CoV-2 specific RNA (E gene)	VIC	None
Internal Control	CY5	None

Note: Collect fluorescent signal at 60℃ step.

4.2.3 Define the general setting:

Sample Volume: 20μL

5. Data Analysis

5.1 QuantStudio™ 5 software

1) Analyze the data using the following Ct settings in QuantStudio™ 5 software.

Unselect the box for "Default Settings", "Automatic Threshold" and "Automatic Baseline".

2) For baseline setting: Use "Baseline Start Cycle 3-15 End Cycle 5-22".

3) For threshold setting: In the "Amplification Plot" display window, manually drag the threshold line until it lies within the exponential phase of the fluorescence curve and above any background signal.

4) Determine the cycle threshold (Ct) values and standard deviation (if applicable) for each assay. Export the run data as an excel file which contains Ct value.

5.2 Bio-Rad CFX-96 software

1) Analyze the data using the settings in Bio-Rad CFX-96 software.

Select FAM, VIC, ROX and CY5 to setting the Baseline Threshold, respectively. Unselect the box for "Auto Calculated".

2) For Baseline Cycles: select "User Defined" to set up "All Selected Rows: Begin 3-15 End: 5-22".

3) For Single threshold setting: manually drag the threshold line until it lies within the exponential phase of the fluorescence curve and above any background signal.

4) Determine the cycle threshold (Ct) values and standard deviation (if applicable) for each assay. Export the run data as an excel file which contains Ct value.

6. Quality control

The Positive Control and Negative Control interpretation guide is summarized in table below:

Control type	ORF1ab (FAM)	N gene (ROX)	E gene (VIC)	Internal control (CY5)
Positive Control	+	+	+	+
Negative Control	-	-	-	-

Note:

"+" means "Detected" ,and the Ct value ≤ 40*, and "-" means "Not Detected", and the Ct value* > 40.

If any of the above controls do not exhibit the expected performance as described in the above table, the test is invalid. The test shall be repeated.

【Threshold and Reference Range】

Through the research on reference values, the Ct reference value of target gene is determined to be 40, the Ct reference value of internal control is determined to be 40.

【Testing Result Interpretation】

The clinical samples interpretation guide is summarized in table below:

Sample ID	ORF1ab （FAM）	N gene （ROX）	E gene （VIC）	Internal control (CY5)	Results	Interpretations
A	+	+	+	+/-	SARS-CoV-2 Positive	SARS-CoV-2 RNA is Detected.
B	+	-	+/-	+/-	SARS-CoV-2 Positive	SARS-CoV-2 RNA is Detected. Missing amplification of individual targets may be due to: 1) the sample concentration is near or below the LoD of the test, 2) a mutation in the specific primer/probe, 3) other factors.
C	-	+	+/-	+/-		
D	+	+	+/-	+/-		
E	-	-	+	+/-	Presumptive positive for SARS-CoV-2	Sarbecovirus RNA is detected but SARS-CoV-2 specific RNA targets are not detected.
F	-	-	-	+	SARS-CoV-2 Negative	SARS-CoV-2 RNA is Not Detected.
G	-	-	-	-	Invalid.	Results are invalid. Repeat test. If the result is still invalid, a new sample should be collected.

Note: "+" means "Detected" ,and the Ct value ≤ 40, and "-" means "Not Detected", and the Ct value＞40.

【Limitations of Detection Method】

- Performance of ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit have only been established in the specimens collected with oropharyngeal swabs.
- Negative results do not preclude infection of SARS-CoV-2 and should not be used as the sole basis for treatment or other patient management decision. Optimum specimen type and timing for peak viral levels during infections caused by SARS-CoV-2 have not be determined. Collection of multiple specimens (types or time point of infection) from the same patient may be necessary to detect the virus.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are in the specimen or if inadequate numbers of organisms are present in the specimen.
- A false positive result may be observed if cross contamination occurred during the specimen handling or preparation.
- If the virus mutates in the RT-qPCR target region, SARS-CoV-2 may not be detected or may be detected less predictively.
- Test performance can be affected because the epidemiology and clinical spectrum of infection caused by SARS-CoV-2 is not fully understand.
- Detective of virus RNA may not indicate the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.
- The performance of this test has not been established for monitoring treatment of SARS-CoV-2 infection.
- The performance of this test has not been established for screening of blood or blood products for the present of SARS-CoV-2.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Missing amplification of the SARS-CoV-2 specific targets may be due to:1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the corresponding target region, or 3) other factors.
- E gene could not distinguish between SARS-CoV-2 and SARS-CoV. Additional confirmatory testing may be conducted if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.

【Product Performance Indicators】

Product analysis performance evaluation results:

1. The analytical sensitivity of this kit is 200 copies/mL.
2. Precision: the precision is expressed in CV% of the Ct values. The CV% of the Ct values of the test kit is all less than 5% for intra-batch precision, inter-batch precision, intra-day precision, inter-day precision, and precision tests between different operators and instruments.
3. Coincidence rate of negative/positive reference materials: The coincidence rates of 3 positive reference materials and 6 negative reference materials all are 100%.
4. Cross-reaction: no cross-reaction with other pathogens such as seasonal influenza A(H1N1) virus, influenza A (H1N1-2009) virus, influenza A H3N2, H5N1, H7N9,influenza B Yamagata, influenza B Victoria, coronavirus (HKU1, OC43, NL63, 229E), parainfluenza 1/2/3/4, Rhinovirus A/B/C, Adenovirus types 1, 2, 3, 4, 5, 7 & 55, Respiratory syncytial virus, enterovirustypes A/B/C/D, EB virus, Measles virus, Human cytomegalovirus, Norovirus, Varicella-zoster virus, rotavirus, Mumps virus, Human metapneumovirus, *Chlamydia pneumonia*, *Mycoplasma*

pneumonia, *Legionella*, *Bacillus pertussis*, *Haemophilus influenza*, *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Klebsiella pneumonia*, *Aspergillus fumigatus*, *Candida albicans*, *Candida glabrata*, *Cryptococcus neoformans* and human genomic DNA that are similar to SARS-CoV-2 or cause similar symptoms.

5. Endogenous substances such as whole blood(5%) and mucus(0.1mg/mL) that may be present in sputum and throat swab specimens have no interference with the test results of the kit.
6. Exogenous interfering substances: the therapeutic drugs against COVID-19 in the patients, such as Benzocaine(1000μg/mL), Oxymetazoline(1μg/mL), Sodium chloride(1000μg/mL), Beclomethasone(1000μg/mL), Dexamethasone(500μg/mL), Flunisolide(10μg/mL), Triamcinolone acetonide(1μg/mL), Budesonide(150μg/mL), Mometasone(1μg/mL), Fluticasone(1μg/mL), Histamine hydrochloride(25μg/mL), α-Interferon(1000μg/mL), Zanamivir(10μg/mL), Ribavirin(300μg/mL), Oseltamivir(75μg/mL), Peramivir(10μg/mL), Hydrochloric acid Adrenaline(10μg/mL), Ritonavir(1μg/mL), Arbidol(200μg/mL), Levofloxacin(750μg/mL), Tobramycin(1.5μg/mL), Azithromycin(500μg/mL), Ceftriaxone(200μg/mL) and Meropenem(20μg/mL) will not interfere with the test results of the kit.
7. The clinical evaluation result: 121 positive samples and 115 negative samples were tested. The positive percent agreement (PPA) between the test reagent and the comparative reagent is 99.18%, the negative percent agreement (NPA) is 100% and the Percent Agreement (PA) is 99.58%, and the Kappa value was calculated as 0.9915.

【Reference】

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W.A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Jan 24.

2. Zhang X, Deng T, Lu J, Zhao P, Chen L, Qian M, Guo Y, Qiao H, Xu Y, Wang Y, Li X, Zhang G, Wang Z, Bian C. Molecular characterization of variant infectious bronchitis virus in China, 2019: Implications for control programmes. Transbound Emerg Dis. 2020 Jan 13, 13477.

3. Algaissi Abdullah,Agrawal Anurodh S,Hashem Anwar M,Tseng Chien-Te K. Quantification of the Middle East Respiratory Syndrome-Coronavirus RNA in Tissues by Quantitative Real-Time RT-PCR.[J]. Methods in molecular biology (Clifton, N.J.), 2020, 2099.

【Disposal】

Dispose of hazardous or biologically contaminated materials according to the practice of your institution.

【Contact and Support Information】

For more information about 3D Biomedicine Sciences & Technologies Co., Limited, please visit our website at http://www.3dmedcare.com or contact at E-mail: ivd-support@3dmedcare.com.

For detailed technical questions regarding the use of the 3D Biomedicine Sciences & Technologies Real-Time qPCR Kits, please contact our Technical Support at E-mail: ivd-support@3dmedcare.com.

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【Symbols】

Symbols	Description	Symbols	Description
CE	CE Mark		Date of Manufacture
	Temperature limit		Manufacturer
LOT	Batch code	REF	Catalogue number
Σ	Contains sufficient for <n> tests		Consult instructions for use
EC REP	EU authorized representative	IVD	In vitro diagnostic medical device
	Use-by date		Keep away from sunlight