NANOHELIX
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Ver. 2212-01

DirectFast™ SARS-CoV-2 multiplex assay [for Research Use Only]

Instruction for Use

Kit contents

DirectFast™ SARS-CoV-2 multiplex assay	
Cat. No.	**DFSCV100 (100rxns)**
Enzyme Mix (SCV2)	0.2ml
Reaction Mix (SCV2)	0.4ml
Oligo Mix (SCV2)	0.4ml
PC (SCV2)	0.05ml
RNase-free Water	1ml
Instructions for Use	1ea

Description

***DirectFast™* SARS-CoV-2 multiplex assay,** a probe-based real-time RT-PCR kit, is designed for 'DIRECT' and 'FAST' detection of SARS-CoV-2 RNA. Crude RNA samples prepared by a lysis buffer (NAExDB) or heating, as well as purified RNA, could be used as templates for this assay kit without compromising detection sensitivity and accuracy. And this kit allows the fast reaction to complete the real-time RT-PCR cycles within one hour (45-50 min for 40 cycles). The target multiplicity (ORF8, N, S genes of SARS-CoV-2) lowers the risk of false-negative results induced by mutations of the viral RNA. Additionally, this assay installs a heat-labile UNG (uracil-N-glycosylase) and dUTP system to prevent carryover contamination.

Application

Direct real-time detection of SARS-CoV-2

Store below -20 ℃

NanoHelix Co., Ltd.

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Quality control

By Nanohelix's ISO 13485-certified quality management system, each lot of ***DirectFast™*** **SARS-CoV-2 multiplex assay** was tested against predetermined specifications to ensure consistent product quality.

Protocol

1. Template (Nucleic acid preparation)

Template nucleic acids can be prepared by one of these procedures.

(a) The commercially available nucleic acid extraction procedures generate highly purified RNA, including PureHelix™ Viral DNA/RNA Kit V2 (Cat No. VNK200D, NanoHelix) and QIAamp® DSP viral RNA mini Kit (Cat No. 61904, QIAGEN). Users should follow the manufacturer's recommendation for use.

(b) A fast and straightforward nucleic acid preparation procedure using the '10x Nucleic acid extraction solution (Cat No. NAExDB, NanoHelix)'.

This procedure can only be applied to the specimens collected in UTM, VTM, TE, or equivalent transport medium. The samples should not contain any protein denaturing agents, like guanidine HCl, guanidine thiocyanate, strong detergents, or alcohols.

① Take 90μL of specimen collected in UTM (universal transport medium or VTM) or TE buffer (10mM Tris, pH 8.0, 1mM EDTA), and place it in a 1.5ml tube.

② Add 10ul of 10X Nucleic acid extraction solution.

③ Mix gently by tapping or vortexing and spin down for a few seconds.

④ Use 3ul as a nucleic acid sample (PCR template) per reaction.

(c) Heating procedure.

This procedure can only be applied to the specimens collected in UTM, VTM, TE, or equivalent transport medium. The samples should not contain any protein denaturing agents, like guanidine HCl, guanidine thiocyanate, strong detergents, or alcohols.

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① Mix well the specimen collected in UTM (universal transport medium or VTM) or TE buffer (10mM Tris, pH 8.0, 1mM EDTA) by vortexing or pipetting.

② Take 50µL of the specimen and place it in a 1.5ml tube. Close the tube cap tightly.

③ Transfer the tube on a dry bath (heat-block) that pre-heated to 92~96°C.

④ After 5 minutes of heating, take out the tube and make cool at room temperature.

⑤ Mix gently by tapping or vortexing, and centrifuge for 2 minutes.

⑥ Use 3µL of the supernatant as a nucleic acid sample (PCR template) per reaction.

2. Reagent preparation

(a) All reagents, stored at -20°C or below, should be entirely thawed at room temperature before use.

(b) All reagents should be used immediately after thawing to reduce the time at room temperature.

(c) Vortex and spin-down steps are necessary when mixing the reagent.

3. PCR Master mix

(a) Prepare the PCR Master mix

The amount of PCR Master mix should be prepared by calculating the overage, corresponding to at least 1~2 reactions more than the number of test and control. (PCR Positive and Negative Control)

Components	Volume of 1 Test
Reaction Mix (SCV2)	**4µL**
Enzyme Mix (SCV2)	**2µL**
Oligo Mix (SCV2)	**4µL**
RNase-free Water	**7µL**
Total volume	**17µL**

(b) Vortex and centrifuge briefly the PCR Master mix.

(c) Add PCR master mix to PCR tubes and add 3µL of the nucleic acid sample.

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- Negative Control (NC) : Add 3µL of RNase free Water instead of the nucleic acid sample.
- Positive Control (PC) : Add 3µL of Positive Control instead of the nucleic acid sample.

Components	Volume of 1 Test
PCR Master mix	**17µL**
Nucleic acid sample , or RNase-free Water for negative control , or PC (SCV2) for positive control	**3µL**
Total volume	**20µL**

(d) Close the PCR tube cap or film, and centrifuge briefly. Then make sure the solution is collected at the bottom of the tube.

4. Set-up and Running of the Instrument

(a) Selection of fluorophores for each target

Instrument	Target gene			
	S	ORF8	N	IC(RNase P)
CFX 96	FAM	HEX	Texas Red	Cy5
ABI 7500(Fast)	FAM	JOE	Texas Red	Cy5

(b) PCR Condition

Step	PCR Condition		Cycle(s)
	CFX 96	ABI 7500(Fast)	
cDNA Synthesis	50℃ for 10 min	50℃ for 10 min	1
Enzyme activation	95℃ for 3 min	95℃ for 3 min	1
PCR Amplification	95 ℃ for 1 sec	95 ℃ for 10 sec	2
	60 ℃ for 20 sec	60 ℃ for 20 sec	
	95 ℃ for 1 sec	95 ℃ for 10 sec	40
	60 ℃ for 5 sec Collect the fluorescence data	60 ℃ for 30 sec Collect the fluorescence data	

5. Result Analysis

(a) Threshold and Base line setting

Instrument	Threshold	Base start-end
CFX 96	S, ORF8, IC(RNaseP) : 300, N : 400	-
ABI 7500(Fast)	All target [S, ORF8, N, IC(RNaseP)] : 20,000	3-15

(b) Interpretation criteria for quality control

Negative and positive control tests should be examined before interpretation of results. If the control test results are invalid, the results cannot be interpreted or reported. Control test results should be interpreted according to the criteria listed in the below table.

Control	Target gene(Fluorescence) and Ct Value				Interpretation
	S(FAM)	ORF8(HEX/JOE)	N(Texas Red)	IC(Cy5)	
PC(SCV2)	< 27	< 27	< 27	< 27	Valid
NC	≥ 37 or N/A	≥ 37 or N/A	≥ 37 or N/A	≥ 37 or N/A	Valid

(c) Interpretation criteria for specimen

① Individual target gene Ct value

Target	Fluorescence	Ct value	Interpretation
S	FAM	< 37	Positive (+)
		≥ 37 or N/A	Negative (-)
ORF8	HEX/JOE	< 37	Positive (+)
		≥ 37 or N/A	Negative (-)
N	Texas Red	< 37	Positive (+)
		≥ 37 or N/A	Negative (-)
IC	Cy5	< 37	Positive (+)
		≥ 37 or N/A	Negative (-)

② Result Interpretation

Case	IC	S	ORF8	N	Interpretation	Comment
1	+/-	+	+	+	SARS-CoV-2 Detected	Result is valid. IC amplification may be inhibited or not confirmed by the dominant amplification of the target.
2	+/-	Two of three +.			SARS-CoV-2 Detected	Result is valid. Missing amplification of individual targets may be due to: • a sample at concentrations near or below the limit of detection of test. • a mutation in the corresponding target region, or other factors.
3	+/-	One of three +.			Inconclusive	Repeat the test. If the result is still inconclusive, It is recommended to perform nucleic acid extraction again or obtain a new specimen.
4	+	-	-	-	SARS-CoV-2 Not detected	Result is valid.
5	-	-	-	-	Invalid/ Retest	Result is invalid. Repeat the test. If the result is still invalid, It is recommended to perform nucleic acid extraction again or obtain a new specimen.

Products

Cat. No.	Products	Size
DFSCV100	*DirectFast™* SARS-CoV-2 multiplex assay [for Research Use Only]	100rxns

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