

HelixDtec™ COVID/Flu/RSV Combo Kit [for Research Use Only]

Kit Contents

HelixDtec™ COVID/Flu/RSV Combo Kit	
Cat. No.	COVFR100 (100tests)
4x CFR Combo Premix	0.5ml
CFR OligoMix	0.5ml
Positive control (CFR)	0.05ml
RNase-free Water	1.0ml
Instructions for Use	1ea

Description

HelixDtec™ COVID/Flu/RSV Combo Kit represents an advanced probe-based multiplex real-time RT-PCR Kit meticulously designed for simultaneous in vitro detection and differentiation of RNA from SARS-CoV-2, influenza A, influenza B, and RSV (respiratory syncytial virus) within a single-tube reaction.

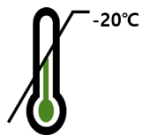
To ensure precise and reliable detection, the kit utilizes distinct probes for two regions of the SARS-CoV-2 virus (RdRP gene and E gene), with each probe labeled with FAM and HEX fluorescence, respectively. Influenza A and B are concurrently detected by Cal-Red 610 labeled probes during the amplification cycles, followed by differentiation through melting curve analysis. RSV A and B genes are collectively amplified and detected by probes labeled with Cy 5.5 fluorescence. The internal control (IC), targeting the human RNase P gene, is detected with a Cy 5-labeled probe.

The provided **4x CFR Combo Premix** contains all the enzymes and buffer components required for the RT-PCR at a 4x concentration. The inclusion of UDG enzyme and dUTP in this premix effectively prevents carryover contaminations.

Application

Combination tests for SARS-CoV-2, Flu A, Flu B and RSV

Storage



Store below -20°C

Shelf life



12 months

Quality Control

By Nanohelix's ISO 13485-certified quality management system, each lot of **HelixDtec™ COVID/Flu/RSV Combo Kit** was tested against predetermined specifications to ensure consistent product quality.

Protocol

1. Reagent preparation

- 1) All reagents, stored at -20°C or below, should be entirely thawed at room temperature before use.
- 2) All reagents should be used immediately after thawing to reduce the time at room temperature.
- 3) Vortex and spin-down steps are necessary when mixing the reagent.

2. PCR master mix

- 1) Prepare the PCR Master mix.

Components	Volume per reaction
Template	5 µL
4x CFR Combo Premix	5 µL
CFR OligoMix	5 µL
RNase-free Water	5 µL
Total volume	20 µL

- 2) Close the PCR tube cap or film, and centrifuge briefly.

3. Instrument Set-up

1) Selection of fluorophores

Instrument	Target				
	SARS-CoV-2		Flu A/B	RSV	IC (RNase P)
	E gene	RdRP			
CFX 96	FAM	HEX	Cal Red 610	Cy5.5	Cy5

2) PCR condition

Step	PCR Condition	Cycle(s)
cDNA Synthesis	50 °C for 10 min	1
Enzyme activation	95 °C for 10 min	1
PCR Amplification	95 °C for 20 sec	2
	60 °C for 20 sec	
	95 °C for 20 sec	45
	60 °C for 10 sec Collect the fluorescence data	
* Melting analysis (for Flu A/B typing)	95°C ~ 55°C (decrement rate: 5s / 0.5°C)	1

*If FluA/B typing is unnecessary, skip this step.

4. Result Analysis

1) Threshold setting

Instrument	Threshold	Base start-end
CFX 96	All target (E gene, RdRp, Flu, RSV, RNase P) : 300	Auto

2) 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 (Fluorescence) and Ct Value					Interpretation
	E gene (FAM)	RdRp (HEX)	Flu (Cal Red 610)	RSV (Cy 5.5)	IC (Cy5)	
Positive control	< 27 Ct					Valid
Negative control	≥ 37 Ct or N/A					Valid

3) Interpretation criteria

① Ct results

Target		Fluorescence	Ct value	Interpretation
SARS-CoV-2	E gene	FAM	< 37	SARS-CoV-2 Positive (+)
	RdRp	HEX/JOE	< 37	
Flu A/B		Cal Red 610	< 37	Flu A/B Positive (+)
RSV		Cy5.5	< 37	RSV Positive (+)
IC		Cy5	< 37	IC Positive (+)

② Tm results (for FluA/B typing)

Target	Fluorescence	Tm	Interpretation
Flu	Cal Red 610	68 ~ 72°C	Flu B
		79 ~ 83°C	Flu A

* Melt peak threshold : 100

③ Result Interpretation

Case	IC (Cy5)	E gene (FAM)	RdRp (HEX)	Flu (Cal Red 610)	RSV (Cy5.5)	Interpretation
1	+	+	+	-	-	SARS-CoV-2
2-1	+	-	-	+ (Tm 79~83°C)	-	Flu A
2-2	+	-	-	+ (Tm 68~72°C)	-	Flu B
3	+	-	-	-	+	RSV
4	+	-	-	-	-	Negative
5	-	+/-	+/-	+/-	+/-	Invalid/ Retest

Product

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COVFR100	HelixDtec™ COVID/Flu/RSV Combo Kit	100tests