

**HelixDtec™ COVID/Flu/RSV Assay Kit** [for Research Use Only]**Kit Contents**

HelixDtec™ COVID/Flu/RSV Assay Kit	
Cat. No.	SCVFR100 (100tests)
5x Reaction Buffer (SCVFR)	0.4ml
Enzyme Mix (SCVFR)	0.2ml
OligoMix (SCVFR)	0.4ml
Positive control (SCVFR)	0.05ml
RNase-free Water	1.0ml
Instructions for Use	1ea

**Description**

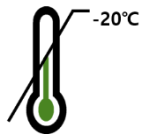
**HelixDtec™ COVID/Flu/RSV Assay Kit** represents an advanced probe-based multiplex real-time RT-PCR kit meticulously designed for simultaneous in vitro detection and differentiation of RNA from SARS-CoV-2, Influenza, and RSV (respiratory syncytial virus) within a single-tube reaction.

To ensure precise and reliable detection, the kit utilizes distinct probes for two regions of the SARS-CoV-2 virus (ORF8 gene and ORF1ab gene), wherein both probes are tagged with HEX fluorescence. Influenza A and B are collectively amplified and detected by probes labeled with Cal-Red 610. RSV A and B genes are collectively amplified and detected by probes labeled with FAM fluorescence. The internal control (IC), targeting the human RNase P gene, is detected with a Cy 5-labeled probe. Additionally, this assay installs a heat-labile UNG (uracil-N-glycosylase) and dUTP system to prevent carryover contamination.

## Application

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

### Storage



Store below -20°C

### Shelf life



12 months

## Quality Control

Each lot of **HelixDtec™ COVID/Flu/RSV Assay 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
5x Reaction Buffer(SCVFR)	4 µL
Enzyme Mix (SCVFR)	2 µL
OligoMix (SCVFR)	4 µL
RNase-free Water	5 µL
<b>Total volume</b>	<b>20 µL</b>

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

### 3. Instrument Set-up

#### 1) Setting of Fluorescence channel

Instrument	Target			
	RSV A/B	SARS-CoV-2	Flu A/B	IC (RNase P)
CFX 96	FAM	HEX/VIC	Cal Red 610	Cy5
ABI7500 (Fast) / QuantStudio 5	FAM	VIC	Texas Red /ROX	Cy5

## 2) PCR condition

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

## 4. Result Analysis

### 1) Threshold setting

Instrument	Threshold	Baseline start	Baseline end
CFX 96	All targets (SARS-CoV-2, RSV, Flu, RNase P) : 300	Auto	Auto
ABI 7500(Fast) /QuantStudio 5	All targets (SARS-CoV-2, RSV, Flu, RNase P) : 20,000	3	15

### 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
	RSV (FAM)	SARS-CoV-2 (HEX/VIC)	Flu (Cal Red 610)	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
RSV	FAM	< 37	RSV Positive (+)
SARS-CoV-2	HEX/VIC	< 37	SARS-CoV-2 Positive (+)
Flu	Cal Red 610	< 37	Flu Positive (+)
IC	Cy5	< 37	IC Positive (+)

② Result Interpretation

Case	IC (Cy5)	RSV (FAM)	SARS-CoV-2 (HEX/VIC)	Flu (Cal Red 610)	Interpretation
1	+	+	-	-	RSV
2	+	-	+	-	SARS-CoV-2
3	+	-	-	+	Flu
4	+	-	-	-	Negative
5	-	+/-	+/-	+/-	Invalid/ Retest

## Products

Cat. No.	Products	Size
SCVFR100	HelixDtec™ COVID/Flu/RSV Assay Kit	100tests