

# COVID / Flu / RSV Simplicity Panel™ 384-Well

## Product Specification Sheet

RUO Product\*



### Product Description:

The COVID/Flu/RSV *Simplicity Panel™* is an *in-vitro* multiplex real-time reverse transcription polymerase chain reaction (rRT-PCR) assay for the qualitative identification of respiratory viral nucleic acids in multiple sample types. This method is highly accurate, analytically sensitive, and is used for the identification of disease-causing organisms by amplifying and detecting genetic material of target pathogens in samples.

#### This panel detects the following targets:

- SARS-CoV-2
- Influenza A including H1N1 and H3N1
- Influenza B (Victoria and Yamagata lineages)
- Respiratory Syncytial Virus (RSV) A and B
- Ribonuclease P (RP)

Product Information	
<b>COVID/ Flu/ RSV <i>Simplicity Panel™</i> (384-well Plate)</b>	
<b>Part Number</b>	P-SABR384-001-A P-SABR384-002-A
<b>Number of Panels</b>	382
<b>Positive Control</b>	SARS-CoV-2/ Flu/ RSV
<b>Storage Temperature</b>	-25°C to -15°C

Product Specifications	
<b>QC Test</b>	qPCR Cycle Threshold Percent CV
<b>Specification</b>	≤ 2.5

QC Results	
<b>Positive</b>	meets specification
<b>Negative</b>	meets specification
<b>Targets</b>	meets specification

#### Disclaimer - Use of PCR and Patent

This product is for basic PCR and is outside of any valid US patents assigned to Hoffman La-Roche.

#### ISO Certification

This product was manufactured in a facility whose Quality Management System is certified as being in conformity with ISO 13485:2016 by Intertek.

#### \* Limitations of Use

For Research Use Only. Not for use in diagnostic procedures.

#### Product Guarantee

This kit has been shown to generate reliable, repeatable and high-performance results.

Please contact Molecular Designs for technical assistance. If not completely satisfied, our team will help you identify and address the issue and replace the assays as needed.

# Usage Information

## ▶ Reagent Storage and Use Guidelines

1. Store all reagents at -25°C to -15°C.
2. Do not freeze-thaw plates more than 3 times.

## ▶ The Following is Included in the Kit:

1. 384-well PCR plate pre-loaded with the multiplex *Simplicity Panel™* assays and positive control. Negative control assay is plated but negative control is user supplied.

## ▶ The Following is Supplied by the User: Materials

1. Extracted Sample(s)
2. qPCR optical film
3. Sealer for optical film
4. Negative Control

## ▶ Equipment

1. Manual defrost -20°C freezer
2. Laminar Flow or PCR Dead Air Box for general plate setup. Do not use Laminar Flow for infectious samples
3. Pipettes and appropriate filtered pipette tips
4. Plate Vortex [recommend Vortex Genie 2 (Model G560) with the 3-inch platform and rubber cover]
5. Plate centrifuge

## ▶ Instrumentation

1. CFX384 Touch Real-Time PCR Detection System (or equivalent)

## ▶ General Guidelines and Safety Precautions

1. As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.
  - a) Do not pipette by mouth.
  - b) Do not eat, drink, or smoke in designated work areas.
  - c) Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples to prevent contamination. Avoid contaminating gloves when handling samples and controls.
  - d) Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
  - e) Thoroughly clean and disinfect all laboratory work surfaces.

**NOTE:** Do not use sodium hypochlorite solution (bleach) to clean up a spill or to disinfect a plate before disposal as it can react with the common extraction reagents and generate toxic byproducts. If spills occur, follow internal procedures to immediately clean and decontaminate the surface of instrument.
2. A laminar flow or PCR Dead Air Box is recommended to reduce contamination probability.
3. The use of filtered, sterile and nuclease-free pipette tips is recommended.
4. False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

# Usage Information

## ▶ Reaction Plate Setup

1. Remove a reaction plate from the -20°C manual defrost freezer.
2. Use the plates within 1 hour of thawing.
3. Spin down the plate for 30 seconds in a plate centrifuge.
4. Carefully remove the foil seal from the plate.
5. Add 4.0 µL of the sample being tested to each of the target wells.  
**NOTE:** 384-well plates have 16 rows (labeled A:P) and 24 columns (labeled 1:24). When plating samples, alternate rows are pipetted to accommodate the use of a multichannel pipette. Refer to page 5 of this document for the detailed well/ panel layout.
6. Do not add any additional liquid to the Positive Control. All components have been added to these wells.
7. Add 4.0 µL of negative control (user provided) to the Negative Control well.
8. Seal the PCR plate using optical qPCR film.
9. Optional: vortex the plate, at least 5 seconds per plate quadrant.
10. Optional: spin down the plate in a plate centrifuge.

## ▶ Procedural Notes

1. Do not reuse consumables. They are for one-time use only.
2. Always use caution when transferring specimens from a primary collection tube to a secondary tube.
3. Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.
4. Always use a new pipette tip for each specimen.
5. For testing of previously frozen sample, ensure samples are equilibrated to room temperature and well mixed prior to use.

## ▶ Target Layout per panel (384-well plate, 382 panels per plate). See Page 5 for the complete layout of a 384-well plate).

1. Each well on the plate contains the COVID/Flu/RSV panel mastermix.
2. Full plate layout:
  - a) The Positive Control is plated in O24.
  - b) The Negative Control is plated in P24.  
Note: No additional liquid needs to be added to the pre-plated PC. However, negative control needs to be added to P24.

*Following fluorophores are used: FAM for SARS-CoV-2; CAL Fluor Orange 560 for Influenza A; CAL Fluor Red 610 for Influenza B; Quasar 670 for RSV and Quasar 705 for Ribonuclease P.*

# Usage Information

## ▶ Real-Time PCR Detection System qPCR Run Setup

1. Open the specified run template and fill in the sample name fields with unique sample IDs corresponding to the samples being processed.
2. **NOTE:** This step can also be done prior to reaction plate setup if sample IDs have already been specified.
3. Place the reaction plate into the instrument in the appropriate orientation (A1 in the upper left corner), close the instrument lid and initiate the run.

## ▶ Thermocycling Protocol

1. Reverse Transcription
  - a) 5 minutes at 50°
2. Denaturation
  - a) 3 minutes at 95°C
3. Annealing and Extension  
40 cycles consisting of:
  - a) 5 seconds at 95°C
  - b) 30 seconds at 60°C, with fluorescence acquisition during this step

## ▶ Amplification Interpretation and Troubleshooting

\* It is recommended to consider Public Health guidance for determining Limit of Detection and a CT cut off for establishing negative results for this rRT-PCR assay.

1. The laboratory should establish cycle threshold (CT) cutoffs as appropriate for their sample workflow and procedures. It is recommended that CT cutoffs are determined during the validation of the test.
2. The laboratory should evaluate the curve shape when considering whether a sample with a given CT should be considered positive:
  - a) Plate sealing issues can lead to jagged curve shapes or rising/decreasing baselines that lead to inaccurate data (erroneous CT value).
  - b) Inappropriate mixing or centrifuging can lead to inaccurate data.
3. If user suspects contamination, it is recommended to clean and disinfect the laboratory area and re-test to ensure proper results.
4. A negative result for the endogenous control can be discarded if the sample is detected positive for a target assay and if the positive and negative controls have proper results.
5. Any failure of the positive or negative control should require a repeat run. If the control failure continues, it is recommended to have the qPCR instrument and the sample extraction workflow evaluated to ensure they are functioning properly.



# DOC-SABR384-INSERT-B (DOC-2349) Ver. 0

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## Approved By:

[\(CO-216\) Updates to 384 well PSS](#)

### Description

Addition of Simplicity Panel TM and Positive control info to the front page. Covid-19 PSS has an additional sentence per Jenny under Limitation of Use. On Page 2, under "following is included in the Kit" Updated info to include Simplicity PanelTM branding, Details for negative control included, Under "following is supplied by user: Materials" #4 Negative control added. On Page 3, edits to "Reaction Plate Setup": #7 edited to delete instruction for Neg Control, #8 added to include instruction for Neg Control, #10 and #11: edited to make these steps Optional inline with client needs, layout target layout title edited for 384 well plates to include ref to only page 5. Plate map on page 5 put in landscape.

### Justification

Updates to PSS to reflect business development and changes.

#### Assigned To:

Lara Self

#### Initiated By:

Lara Self

#### Priority:

Medium

#### Impact:

Minor

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## Version History:

Author	Effective Date	CO#	Ver.	Status
Lara Self	June 7, 2023 3:45 PM CDT	<a href="#">CO-216</a>	0	Published