molecular designs

Product Specification Sheet

DOC-SABR096-INSERT-A

Product Description:

The COVID/Flu/RSV Panel is a 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 a highly accurate, extremely sensitive and, is used for 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)
- RSV A and B
- Ribonuclease P

Product Information	
COVID/ Flu/ RSV Panel Plate, 0.1mL, 96-well, Clear Breakaway	
Part Number	P-SABR096-001-A
Number of Reactions	94
Storage Temperature	-25°C to -15°C

Product Specifications	
QC Test	qPCR Cycle Threshold Percent CV
Specification	≤ 2.5

QC Results	
Positive	meets specification
Negative	meets specification
Targets	meets specification

Limitations of Use

Molecular Designs products are for Research Use Only and intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

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.

Product Guarantee

This kit is proven in PCR and results in reliable, repeatable and high-performance results. Please contact Molecular Designs for technical assistance. If not completely satisfied, we will send you a free of charge replacement.

Jaspreet Seth

Jaspreet Seth
Vice President, Quality

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. 96-well PCR plate pre-loaded with the multiplex assay mix
2. Positive Control tubes containing pooled plasmids
3. Negative Control containing nuclease-free water

The Following is Supplied by the User: Materials

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

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
6. Lab utility knife

Instrumentation

1. CFX96 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 Dear 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.

Please contact Molecular Designs for more information
2 Perimeter Park, Birmingham, AL 35243
www.moleculardesigns.com

Usage Information

Reaction Plate Setup

1. Remove a reaction plate from the -20°C manual defrost freezer.
2. If not running 94 samples, determine the number of samples that will be used from the reaction plate, score the foil seal on the PCR break-away plates using the lab utility knife and tear the plate along the perforated edge between samples wells to obtain the number of panels needed. Ensure the excess panels on the plate are properly labelled and promptly place the remainder back in the -20 °C freezer.
3. Use the plates within 1 hour of thawing, keep sealed and store refrigerated at 4°C if not using immediately.
4. Spin down the plate for 30 seconds in a plate centrifuge.
5. Carefully remove the foil seal from the plate.
6. Add 4.0 µL of the sample being tested to each of the target wells.
7. Do not add any additional liquid to the Positive Control and Negative Control wells. All components have been added to these wells.
8. If using a partial plate add 4.0 µL positive control to the positive control well. This is not needed for the initial run because positive control comes preloaded in A1.
9. If using a partial plate, add 4.0 µL nuclease-free water to the negative control well. This is not needed for the initial run because negative control comes preloaded in B1.
10. Seal the PCR plate using optical qPCR film. Note: If using a partial plate, remove the excess optical seal using the utility knife and ensure the plate is sealed.
11. Vortex the plate, at least 5 seconds per plate quadrant.
12. 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.

Plate Layout (94Panels/ 96 well plate)

1. Each well on the plate contains the COVID/Flu/RSV panel mastermix.
2. If using full plate:
   a) The Positive Control is plated in A1.
   b) The Negative Control is plated in B1. Note: No additional liquid needs to be added to the pre-plated PC and NC
3. If using partial plates, positive and negative controls can be prepared by plating 4 µL of the positive control and 4 µL of the negative control in the wells chosen.
4. We recommend that positive is setup on the left top and next to it negative is setup.

Following fluorophores are used: FAM for SAR-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

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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.
   Note: This step can also be done prior to reaction plate setup if sample IDs have already been specified.
2. 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.

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