# TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Panel

## USER GUIDE

Multiplex real-time PCR assay for the detection and differentiation of SARS-CoV-2, influenza A, influenza B, and RSV A/B RNA

**Catalog Number** A59526

**Publication Number** MAN0029779

**Revision** A.0



ThermoFisher SCIENTIFIC

Life Technologies Corporation | 6055 Sunol Blvd | Pleasanton, California 94566 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

**Revision history: MAN0029779 A.0 (English)**

| Revision | Date | Description |
|---|---|---|
| A.0 | 30 August 2023 | New document for the TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Panel. |

The information in this guide is subject to change without notice.

# Contents

# 1 TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Panel product information



## Product description

The TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Panel is a multiplexed real-time PCR assay for research on nucleic acids from SARS-CoV-2, influenza A, influenza B, RSV, and RNase P in a single reaction well.

Each kit includes the following components:

- TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Panel
  - TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Assay—Multiplex assays that contain primer and probe sets specific to the following targets:
    - Three SARS-CoV-2 targets (Orf1a, Orf1b, and N genes)
    - Two influenza A targets (PB1 & M genes)
    - Two influenza B targets (M & NS genes)
    - Two RSV A targets and one RSV B target (NP, M, and L protein genes)
    - RNase P (human sample collection control)
- TrueMark™ 1-Step Select Master Mix (No ROX)—Ready-to-use PCR mix, including reverse transcriptase, polymerase, dNTPs, salt, and buffer.

The following controls are recommended for this assay:

- AcroMetrix™ Multi-Analyte SARS-CoV-2, Flu A/B, RSV A/B Control—Inactivated viral control that contains SARS-CoV-2, influenza A, influenza B, and RSV (positive control)
- Nuclease-free water (not DEPC-treated)

# Contents and storage

**Table 1 TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Panel, 200 reactions (Cat. No. A59526)**

| Component | REF[1] (box REF) | Amount | Storage |
|---|---|---|---|
| TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Assay | A59489 | 2 × 150 µL | –30°C to –10°C |
| TrueMark™ 1-Step Select Master Mix (No ROX) | A59585 | 2 × 1 mL | –30°C to –10°C |

[1] Individual components cannot be ordered separately.

# Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

| Item | Source |
|---|---|
| **Real-time PCR instrument, one of the following:** | |
| Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 96–well 0.2-mL block<br>(used with QuantStudio™ Design and Analysis Desktop Software v1.5.2 or later) | A28139 (instrument only)<br>A28569 (with laptop computer)<br>A28574 (with tower computer) |
| Applied Biosystems™ QuantStudio™ 5 Dx Real-Time PCR Instrument, 96–well 0.2-mL block (RUO mode only) | A47326 (with laptop computer)<br>A47327 (with tower computer) |
| **Software, one of the following:** | |
| QuantStudio™ Design and Analysis v2.7 or later (for use only with the QuantStudio™ 5 Real-Time PCR Instrument) [1] | **thermofisher.com/qpcrsoftware** |
| Applied Biosystems™ Diomni™ Software v4.0 or later (RUO workspace only) | Contact your local sales office |
| **Equipment** | |
| Laboratory freezers, –30°C to –10°C | MLS |
| Refrigerator (2–8°C) | MLS |
| BSL-2 biological safety cabinet | MLS |
| Centrifuge, with a rotor that accommodates standard microplates | MLS |
| Microcentrifuge | MLS |
| Laboratory mixer, vortex or equivalent (capable of mixing speeds of 10,000 rpm or higher) | MLS |

*(continued)*

| Item | Source |
|---|---|
| Single and/or multichannel adjustable pipettors (1.00 µL to 1,000.0 µL) | MLS |
| Cold block (96-well) or ice | MLS |
| **Nucleic acid extraction system and materials *(recommended)*** | |
| KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head | 5400630 |
| KingFisher™ Flex 96 Deep-Well Heating Block | 24075430 |
| KingFisher™ 96 Deep-Well Plate | 95040450, A48305, A48424, 95040455 |
| 96-well plate for the tip comb, one of the following:<br>• KingFisher™ 96 KF microplate<br>• Tip Comb Presenting Plate for KF 96<br>• Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom<br>• Nunc™ MicroWell™ 96-Well Microplate, barcoded<br>• Abgene™ 96-Well Polypropylene Storage Microplate<br>• Abgene™ 96-Well 1.2-mL Polypropylene Deepwell Storage Plate<br>• Nunc™ F96 MicroWell™ Black Polystyrene Plate<br>• Nunc™ F96 MicroWell™ White Polystyrene Plate<br>• KingFisher™ 96 Deep-Well Plate | • 97002540<br>• 267600<br>• 167008<br>• 269787<br>• AB0796<br>• AB1127<br>• 137101<br>• 136101<br>• 95040450, A48305, A48424, 95040455 |
| KingFisher™ 96 tip comb for deep-well magnets | 97002534, A48438, A48414 |
| **Kits and reagents** | |
| MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit | A48383R |
| *(Recommended)* AcroMetrix™ Multi-Analyte SARS-CoV-2, Flu A/B, RSV A/B Control | 954516 |
| Fisher BioReagents™ Ethanol, Absolute, Molecular Biology Grade[2], or equivalent | BP2818100, BP2818500, BP28184 |
| 80% Ethanol, Molecular Biology Grade, Thermo Scientific™ | T08204K7CS |
| Nuclease-free water (not DEPC-treated) | AM9938 (1 x 100 mL)<br>AM9932 (1 x 1,000 mL) |
| **Tubes, plates, and other consumables** | |
| MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL | • 4306737 (with barcode)<br>• 4326659 (with barcode)<br>• N8010560 (without barcode)<br>• 4316813 (without barcode) |
| MicroAmp™ Optical Adhesive Film | 4311971, 4360954 |

*(continued)*

| Item | Source |
|---|---|
| MicroAmp™ Clear Adhesive Film | 4306311 |
| MicroAmp™ Optical Film Compression Pad | 4312639 |
| Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL) | thermofisher.com/plastics |
| Sterile aerosol barrier (filtered) pipette tips | thermofisher.com/pipettetips |
| Reservoir for multichannel pipettes | MLS |

[1] Use of QuantStudio™ Design and Analysis v2.7 or later is recommended for data analysis. It is the responsibility of the laboratories using the assay to design and validate their own experimental design and analysis parameters.

[2] Available at fisherscientific.com.

# Manage software files

## Obtain the assets

To perform data analysis and results interpretation, you must use one of the following software:

- QuantStudio™ Design and Analysis v2.7 or later
- Applied Biosystems™ Diomni™ Software v4.0 or later (RUO workspace only)

**Note:** For information on software installation, security, audit, and e-signature functions, see “Related documentation” on page 31.

Obtain the asset files from https://www.thermofisher.com/order/catalog/product/A59526.

To perform data analysis using QuantStudio™ Design and Analysis v2.7, the Auto Spectral Adjustment (ASA) file must be installed, and the **Reduce dye signal crosstalk by algorithm (it may slow down your analysis)** checkbox selected (see “Analyze the data” on page 26):

**Note:** The ASA algorithm decreases spectral crosstalk. This reduction enables lower thresholds and reduces the potential for false positives and negatives.

| Instrument | Auto spectral adjustment file |
|---|---|
| QuantStudio™ 5 Real-Time PCR Instrument | `asa-v1.4-r5.cfg` |

To perform data analysis using the Diomni™ Software v4.0 or later (RUO workspace only), install the following appropriate assay definition file (ADF) for your instrument:

| Instrument | Assay definition file |
|---|---|
| QuantStudio™ 5 Real-Time PCR Instrument | `SC2-Flu-RSV-Select-RUO_QS5_9602_1.0.0.adf` |
| QuantStudio™ 5 Dx Real-Time PCR Instrument (RUO mode only) | `SC2-Flu-RSV-Select-RUO_QS5Dx_9602_1.0.0.adf` |

## Import and edit the assay definition file

Your SAE user account must have the SAE permission of **Import ADF**.

**Note:** An assay definition file cannot be installed if the regulatory label does not match the workspace.

An assay definition file defines the configuration for a real-time PCR assay for a specific real-time PCR instrument and block type. The ADF contains the analysis settings used for data analysis in the interpretive software.

1. Log in to the Diomni™ Software and enter the Assay Development workspace.
2. In the upper-right corner of the tab, click **Import**.
3. Click **Choose file**, then navigate to and select the file to import in ADF file format.

| Instrument | Assay definition file [1] |
|---|---|
| QuantStudio™ 5 Real-Time PCR Instrument | `SC2-Flu-RSV-Select-RUO_QS5_9602_1.0.0` |
| QuantStudio™ 5 Dx Real-Time PCR Instrument (RUO mode only) | `SC2-Flu-RSV-Select-RUO_QS5Dx_9602_1.0.0` |

[1] Only one matching ADF is required to analyze a data file.

4. Click **Open**.
   The assay definition file is added.
5. Open the imported assay definition file by clicking on the **Assay Definition ID**.
6. In the opened assay definition file, select the **Targets** tab, then click **Edit**.
7. In the **Passive reference** pane, verify that the passive reference is set to **NONE**.
8. In the **Targets** pane, define the targets.
   Refer to Table 2 for target information.

**Table 2 Target, reporter dye, and quencher for the TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Panel**

| Target | Reporter dye | Quencher |
|---|---|---|
| **COV19** | VIC | None |
| **FLUA** | FAM | |
| **FLUB** | ABY | |
| **RSVAB** | ALEXA 647 | |
| **RNASEP** | JUN | |

**IMPORTANT!** The reporter dye is case sensitive. Name the dye as **ALEXA 647** during instrument calibration. The name must contain capital letters. There must be exactly one space between the name and number. Ensure there are no additional spaces.

- Click + **Add target** to add a new target.

- Enter or select the following information.
  - Name
  - Reporter—Select the reporter from the **Reporter** dropdown list.
  - Quencher—Select the quencher from the **Quencher** dropdown list.
  - Toggle the **Internal control** button on or off—If enabled, this identifies the internal control target.
  - Color—Click the color to open the color picker, then select a color. The color is not related to the dye. It is to visualize the targets in the plate layout view.
- Select the target or the targets, then click **Delete** ▸ **OK** to delete the target or the targets.

9. In the **Run method** tab, verify the following run details:

**Table 3 QuantStudio™ instrument thermal protocol**

| Step | Temperature | Time | Number of cycles | Ramp rate |
|---|---|---|---|---|
| UNG incubation | 25°C | 2 minutes | 1 | 1.6°C per second |
| Preincubation | 53°C | 10 minutes | 1 | 1.6°C per second |
| | 85°C | 10 minutes | 1 | 1.6°C per second |
| Activation | 95°C | 2 minutes | 1 | 1.6°C per second |
| Denaturation | 95°C | 3 seconds | 43 | 1.6°C per second |
| Anneal / extension | 60°C | 32 seconds | | 1.6°C per second |

**Table 4 PCR filters for the QuantStudio™ instruments using the TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Panel**

| | | Emission Filter | | | | | |
|---|---|---|---|---|---|---|---|
| | | m1(520±15) | m2(558±11) | m3(586±10) | m4(623±14) | m5(682±14) | m6(711±12) |
| Excitation Filter | x1(470±15) | ✔ | ✔ | ✔ | | | |
| | x2(520±10) | | ✔ | ✔ | ✔ | | |
| | x3(550±11) | | | ✔ | ✔ | | |
| | x4(580±10) | | | | ✔ | ✔ | |
| | x5(640±10) | | | | | ✔ | |
| | x6(662±10) | | | | | | |

For more information on assay definition files, see “Related documentation” on page 31.

10. In the **Primary analysis** tab, select automatic baseline with a start cycle of 5 and an end cycle of auto. Alternatively, base your selection on laboratory validation.

**Note:** Set the appropriate threshold values for each target, as established by your laboratory.

**IMPORTANT!** Do not use automatic threshold values.

11. Determine $C_q$ cutoff values for each target for samples and controls.

    **Note:** The $C_q$ values are equivalent to $C_t$ values.

12. Click **Save**.

## Export and install the assay definition file

Your SAE user account must have the SAE permission of **Export ADF**.

**Note:** An assay definition file cannot be installed if the regulatory label does not match the workspace.

1. Export the assay definition file:
   a. In the Assay Development workspace, select the **RUO** tab.
   b. Click the assay definition file ID to open the file.

| Instrument | Assay definition file [1] |
|---|---|
| QuantStudio™ 5 Real-Time PCR Instrument | SC2-Flu-RSV-Select-RUO_QS5_9602_1.0.0 |
| QuantStudio™ 5 Dx Real-Time PCR Instrument (RUO mode only) | SC2-Flu-RSV-Select-RUO_QS5Dx_9602_1.0.0 |

[1] Only one matching ADF is required to analyze a data file.

   c. In the upper-right corner of the file, click **More actions** ▸ **Export**.
   The file is exported in ADF file format.

2. Install the assay definition file:

   **IMPORTANT!** Ensure the ADF has the target information entered in step 8 on page 9.

   a. Log in to the Diomni™ Software and enter the **RUO** workspace.
   b. In the upper-right corner of the Diomni™ Software RUO workspace menu bar, select **(Settings)** ▸ **Assay definitions**.
   c. In the upper-right corner of the tab, click **Install**.
   d. Click **Accept** to acknowledge the terms of the RUO ADF.
   e. Navigate to, then select the .adf file to import in assay definition file format.
   f. Select the assay definition file for the instrument used for the real-time PCR run.
   The assay definition file is added to the **Assay Definitions** tab.

# 2 Methods

# In-use reagent stability

**Note:** For AcroMetrix™ Multi-Analyte SARS-CoV-2, Flu A/B, RSV A/B Control stability information, see the AcroMetrix™ Multi-Analyte SARS-CoV-2, Flu A/B, RSV A/B Control Package Insert (see "Related documentation" on page 31).

| Reagent | Stability information |
|---|---|
| TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Assay | Once thawed, the TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Assay is stable for a cumulative total of 72 hours at 2–8°C.<br>Do not exceed 4 freeze-thaw cycles. |
| TrueMark™ 1-Step Select Master Mix (No ROX) | Once thawed, the TrueMark™ 1-Step Select Master Mix (No ROX) is stable for a cumulative total of 72 hours at 2–8°C.<br>Do not exceed 4 freeze-thaw cycles. |

# Extract RNA (recommended method)

**IMPORTANT!** Laboratories are responsible for validating their own experimental design, including RNA extraction.

The MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit is recommended for extraction.

## General laboratory recommendations

- Implement standard operating procedures in your laboratory to prevent contamination, such as the following:
  - Frequent glove changes
  - Frequent decontamination of surfaces, equipment, and pipettes with fresh 10% bleach or decontamination solution, followed by 70% ethanol
  - Use of ultraviolet light during biosafety cabinet decontamination (when available)
- Samples should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- To prevent degradation, keep master mixes, assays, and controls on ice or in cold blocks while in use.
- Do not freeze-thaw reagents more than the permitted amount to ensure reliable performance (see "In-use reagent stability" on page 13).
- Aliquot reagents using low nucleic acid binding tubes to prevent stock contamination and reduce the number of freeze-thaw cycles.
- After each run, review the amplification curves for signs of inadequate vortexing or centrifugation.
- To ensure reliable performance of the real-time PCR instrument, perform preventive maintenance according to the instructions provided by the manufacturer in the instrument documentation (see "Related documentation" on page 31).

## Before you begin

**IMPORTANT!** The binding bead mix is not compatible with bleach. For more information, see the SDS.

**Note:** During the wash steps, the wash solution may develop inert white or brown particulates that float in solution. This is not a cause for concern and does not negatively affect performance.

- Extract RNA from 200 µL of sample.
- Determine the number of required reactions based on the number of samples, plus one positive control and one negative control per plate.
- Use 80% Ethanol, Molecular Biology Grade, Thermo Scientific™ for the required number of reactions, sufficient for 1 mL per reaction, plus 10% overage.
- Label the short side of each KingFisher™ 96 Deep-Well Plate (4):

| Label | Number of plates |
|---|---|
| Sample plate | 1 |
| Wash 1 | 1 |
| Wash 2 | 1 |
| Elution plate | 1 |

- Label the short side of the KingFisher™ 96 KF microplate (1):

| Label | Number of plates |
|---|---|
| Tip comb | 1 |

**Note:** The following items can be used to hold the tip comb instead of the KingFisher™ 96 KF microplate:

- Tip Comb Presenting Plate for KF 96
- Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom
- Nunc™ MicroWell™ 96-Well Microplate, barcoded
- Abgene™ 96-Well Polypropylene Storage Microplate
- Abgene™ 96-Well 1.2-mL Polypropylene Deepwell Storage Plate
- Nunc™ F96 MicroWell™ Black Polystyrene Plate
- Nunc™ F96 MicroWell™ White Polystyrene Plate
- KingFisher™ 96 Deep-Well Plate

## Set up the instrument

1. Ensure that the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head is set up with the KingFisher™ Flex 96 Deep-Well Heating Block.

   **IMPORTANT!** Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.

2. Ensure that the **MVP_2Wash_200_Flex** program has been downloaded from the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit product page at **www.thermofisher.com** and loaded onto the instrument.

## Prepare the processing plates

Prepare the processing plates according to the following table. Cover the plates with a temporary seal (such as MicroAmp™ Clear Adhesive Film), then store at room temperature for up to 1 hour while you set up the sample plate.

| Plate ID | Plate position | Plate type | Reagent | Volume per well |
|---|---|---|---|---|
| Wash 1 Plate | 2 | KingFisher™ 96 Deep-Well Plate | Wash Solution | 500 µL |
| Wash 2 Plate | 3 | | 80% Ethanol | 1,000 µL |
| Elution Plate | 4 | | Elution Solution | 50 µL |
| Tip Comb Plate | 5 | Place a KingFisher™ 96 tip comb for deep-well magnets in a KingFisher™ 96 KF microplate | | |

**Note:** The following items can be used to hold the tip comb instead of the KingFisher™ 96 KF microplate:

- Tip Comb Presenting Plate for KF 96
- Nunc™ MicroWell™ 96-Well Microplate, Flat Bottom
- Nunc™ MicroWell™ 96-Well Microplate, barcoded
- Abgene™ 96-Well Polypropylene Storage Microplate
- Abgene™ 96-Well 1.2-mL Polypropylene Deepwell Storage Plate
- Nunc™ F96 MicroWell™ Black Polystyrene Plate
- Nunc™ F96 MicroWell™ White Polystyrene Plate
- KingFisher™ 96 Deep-Well Plate

## Prepare binding bead mix

Prepare the required amount of binding bead mix on each day of use.

1. Vortex the binding beads to ensure that the bead mixture is homogeneous.
2. For the number of required reactions, prepare the binding bead mix according to the following table:

| Component | Volume per well[1] |
|---|---|
| Binding solution | 265 µL |
| Binding beads | 10 µL |
| **Total volume per well** | **275 µL** |

[1] Include 10% overage when preparing the binding bead mix for use with multiple reactions.

3. Mix well by inversion, then store at room temperature.

## Prepare sample plate

1. Invert the binding bead mix 5 times gently to mix, then add 275 µL to each well of the plate.

**Note:** Remix the binding bead mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The binding bead mix is viscous, so pipet slowly to ensure that the correct amount is added. DO NOT reuse pipette tips to add binding bead mix to the samples, as the high viscosity will cause variations in the volumes added.

2. Add 200 µL of sample to each sample well.
3. Add 200 µL of AcroMetrix™ Multi-Analyte SARS-CoV-2, Flu A/B, RSV A/B Control to the positive control well.
4. Add 200 µL of nuclease-free water (not DEPC-treated) to the negative control well.
5. Add 5 µL of Proteinase K to each well in the KingFisher™ 96 Deep-Well Plate labeled "sample plate", including the positive and negative control wells.

## Process the samples

1. Select the **MVP_2Wash_200_Flex** on the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head.
2. Start the run, then load the prepared plates into position when prompted by the instrument.
3. After the run is complete (~22 minutes after start), immediately remove the elution plate from the instrument, then cover the plate with MicroAmp™ Clear Adhesive Film.

**IMPORTANT!** To prevent evaporation, seal the plate containing the eluate immediately.

The samples are eluted in 50 µL of elution solution (see “Prepare the processing plates” on page 15).

**Note:**

- Significant bead carry over may adversely impact RT-PCR performance. If bead carry over is observed, re-extract a new aliquot of the sample.
- To ensure reliable performance of the KingFisher™ Flex Magnetic Particle Processor, perform preventive maintenance as instructed by the manufacturer.

Place the elution plate on ice for immediate use in real-time RT-PCR.

# Prepare real-time PCR reactions

## Guidelines for real-time PCR

**IMPORTANT!**

- Prepare the real-time PCR plate on ice or a cold block. Keep the real-time PCR plate on ice or a cold block until it is loaded into the real-time PCR instrument.
- Run the real-time PCR plate immediately. If the assembled reaction plate is not loaded into the real-time PCR instrument immediately, the plate is stable up to 1 hour at 4°C with protection from light. Failure to do so could result in degraded samples.
- To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Do not use the same pipette for controls and samples, and always use aerosol barrier pipette tips.
- Protect assays from light.
- Keep samples and components on ice or a cold block during use.
- For each real-time PCR plate, include the following controls:
  - Positive control
  - Negative control

## Prepare the real-time PCR reactions

1. If frozen, thaw the reagents on ice or on a cold block.
2. Gently vortex the reagents, then briefly centrifuge the tube or swirl the bottle to collect the liquid at the bottom of the container. Keep on ice until use.

3. Prepare the reaction mix:
   a. For each 96-well plate, combine the following components sufficient for the number of samples plus the positive control and negative control.

| Component | Volume per sample or control | Volume for *n* reactions (including samples and controls) [1] |
|---|---|---|
| TrueMark™ 1-Step Select Master Mix (No ROX) | 6.25 µL | 7.5 x (*n* + 2) µL |
| TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Assay | 1.25 µL | 1.5 x (*n* + 2) µL |
| Nuclease-free water (not DEPC-treated) | 4.5 µL | 5.4 x (*n* + 2) µL |
| **Total reaction mix volume** | **12 µL** | **—** |

[1] All volumes include 20% overage for pipette error.

4. Set up the reaction plate, according to the following:

   **Note:** Prepare the plate on ice or a cold block.

   a. Pipet 12 µL of the reaction mix prepared in step 3 to each well of a MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL.

   b. Add 13 µL of the extracted substances to the designated wells: RNA sample, positive control, and negative control.

   c. Seal the plate thoroughly with MicroAmp™ Optical Adhesive Film.

   **IMPORTANT!**
   - Use ONLY MicroAmp™ Optical Adhesive Film (Cat. No. 4311971, 4360954).
   - DO NOT use optical caps, MicroAmp™ Clear Adhesive Film (Cat. No. 4306311), or any other film or sealing method.
   - DO NOT heat seal the plate.
   - When applying the MicroAmp™ Optical Adhesive Film, ensure that pressure is applied across the entire plate and that there is a tight seal across every individual well. Failure to do so runs the risk of an improperly sealed well, leading to potential well-to-well contamination during vortexing and evaporation during PCR.

   d. Vortex the plate on a flat rubber surface at the highest setting speed for 30-60 seconds as follows: 5-10 seconds in the center of the plate, 5-10 seconds in each plate corner, and 5-10 seconds, again in the center.

   **IMPORTANT!** Move the plate around to ensure equal contact of each part of the plate with the vortex mixer platform.

5. Centrifuge the reaction plate for 1-2 minutes at ≥1,400 × *g* to remove bubbles and to collect the liquid at the bottom of the reaction plate.

6. Check the reaction plate wells for any bubbles or droplets on the sides or adhesive film. Ensure the plate is fully horizontal when doing so and not tilted. If bubbles are observed, repeat step 5.

**Note:** The presence of bubbles can adversely impact PCR performance.

**IMPORTANT!**

- Keep the real-time PCR reaction plate at 2–8°C with protection from light until it is loaded into the real-time PCR instrument immediately before starting the run.
- Run the real-time PCR reaction plate within an hour after preparation. Failure to do so could result in degraded samples.

If using QuantStudio™ Design and Analysis Desktop Software v1.5.2 or later, proceed to “Set up and run the real-time PCR instrument using QuantStudio™ Design and Analysis Desktop Software v1.5.2 or later” on page 20.

If using Diomni™ Software v4.0, proceed to “Create and run the real-time PCR run using Diomni™ Software v4.0 or later (RUO workspace only)” on page 22.

# Set up and run the real-time PCR instrument using QuantStudio™ Design and Analysis Desktop Software v1.5.2 or later

Ensure that your real-time PCR instrument is calibrated for the dyes listed in Table 7. See your instrument user guide for more information.

Create an assay template (EDT) to run the QuantStudio™ 5 Real-Time PCR Instrument using QuantStudio™ Design and Analysis Desktop Software v1.5.2 or later.

1. In the QuantStudio™ Design and Analysis Desktop Software home screen, in the **New Experiment** box, select **Create New Experiment ▸ Template**.

2. In the **Properties** tab, enter or confirm the following.
   - **Name**: Enter a unique name
   - **Instrument type**: **QuantStudio™ 5 System**
   - **Block type**: **96-Well 0.2-mL Block**
   - **Experiment type**: **Standard Curve**
   - **Chemistry**: **TaqMan™ Reagents**
   - **Run Mode**: **Standard**

3. In the **Run Method** tab, set up the thermal protocol for your instrument.
   - **Reaction Volume**: **25 µL**
   - **Heated Cover Temperature**: **105.0°C**

**Table 5 QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block**

| Step | Temperature | Time | Number of cycles | Ramp rate |
|---|---|---|---|---|
| UNG incubation | 25°C | 2 minutes | 1 | 1.6°C per second |
| Preincubation | 53°C | 10 minutes | 1 | 1.6°C per second |
| | 85°C | 10 minutes | 1 | 1.6°C per second |
| Activation | 95°C | 2 minutes | 1 | 1.6°C per second |
| Denaturation | 95°C | 3 seconds | 43 | 1.6°C per second |
| Anneal / extension | 60°C | 32 seconds | | 1.6°C per second |

4. Set up the optical filters for your instrument based on the table below.

**Table 6 PCR filters for the QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block using the TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Panel**

| | | Emission Filter | | | | | |
|---|---|---|---|---|---|---|---|
| | | m1(520±15) | m2(558±11) | m3(586±10) | m4(623±14) | m5(682±14) | m6(711±12) |
| Excitation Filter | x1(470±15) | ✔ | ✔ | ✔ | | | |
| | x2(520±10) | | ✔ | ✔ | ✔ | | |
| | x3(550±11) | | | ✔ | ✔ | | |
| | x4(580±10) | | | | ✔ | ✔ | |
| | x5(640±10) | | | | | ✔ | |
| | x6(662±10) | | | | | | |

5. In the **Plate Setup** tab, confirm that the **Passive Reference** is set to **None**.

6. In the **Targets** table, confirm that the targets, reporter dyes, and quencher are listed correctly with the associated panel.

**IMPORTANT!** The reporter dye names are case sensitive. Name the dyes exactly as listed in Table 7.

**Table 7 Target, reporter dye, and quencher for the TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Panel**

| Target | Reporter dye | Quencher |
|---|---|---|
| **COV19** | VIC | None |
| **FLUA** | FAM | |
| **FLUB** | ABY | |
| **RSVAB** | ALEXA 647 | |
| **RNASEP** | JUN | |

7. Confirm that the targets in step 6 are assigned to each well in the plate layout.

8. Save the plate file and load onto the instrument.

9. Load the plate and place a MicroAmp™ Optical Film Compression Pad with the gray side down on the surface of the real-time PCR reaction plate. Ensure there is a proper seal between the thermal cycler and the adhesive film.

**IMPORTANT!**

- Be careful to place the compression pad with the brown side up and the gray side down, centered on top of the plate.
- Ensure the compression pad is free from wrinkles and signs of deterioration prior to use.
- Each compression pad may be used up to 20 times before discarding. Do not use more than 20 times. Use of deteriorated compression pads may lead to volume leakage and failed reactions.

10. Touch **Start Run**. When prompted, confirm that you inserted a plate.

11. Proceed to "Analyze the data" on page 26.

# Create and run the real-time PCR run using Diomni™ Software v4.0 or later (RUO workspace only)

**IMPORTANT!** Once initiated in Diomni™, workflows cannot be transferred to Design and Analysis software. The workflow must be completed entirely with Diomni™ in the RUO workspace only.

There are three steps to create a qPCR run.

- Configure the plate (see page 22)
- Add the reagents (see page 23)
- Save the plate setup (see page 23)

Your SAE user account must have the SAE permission of **Create Run**.

## Configure the plate

An RUO assay definition file must be installed to add a run in the RUO workspace (see "Import and edit the assay definition file" on page 9).

1. In the RUO workspace, select the **qPCR** tab.

2. In the upper-right corner of the tab, click **Create qPCR run**.

3. Select the instrument and the block type, then click **Save**.

   The instruments that are displayed in the **Select instrument and block type** dialog box match the regulatory label for the workspace.

4. Select the appropriate assay definition file, then click **Confirm**.

   **IMPORTANT!** You must use the ADF provided by Thermo Fisher™ to ensure optimal analysis and algorithm use during data analysis.

   **Note:** Enter or confirm target information in the ADF (see Table 2).

5. *(Optional)* Import samples (see "Related documentation" on page 31).

6. Select a well, then enter or select the following information.
   - A sample or control name in the **Sample** field.
     The assay is populated in the **Assay** dropdown list.
   - *(Optional)* A comment in the **Comment** field.

Select one of the following options.

- Click **Next** to proceed to adding reagents.
- Click **Save for later**. The **qPCR** tab is displayed. The saved run is included in the list of qPCR runs.

## *(Optional)* Add reagents

1. Toggle **Use barcode scanner** on or off.
2. If barcode scanning is enabled, scan the barcode of the reagent.
   Do not manually edit the fields if the barcode of the reagent was scanned.
3. If barcode scanning is not enabled, click **Add reagent**, then enter the following information.

   **Note:** To import reagents from a CSV file, click **Import reagents**. To export reagents, click **Export reagents**.

   - Enter the barcode in the **Barcode** field
   - Enter the reagent name in the **Reagent name** field
   - Enter the part number of the reagent in the **Part number** field
   - Enter the lot number of the reagent in the **Lot number** field
   - Enter the expiry date of the reagent in the **Expiry date** field

Select one of the following options.

- Click **Next** to proceed to saving the plate setup.
- Click **Save for later**. The **qPCR** tab is displayed. The saved run is included in the list of qPCR runs.

## Save the plate setup

In order to start the run, your SAE user account must have the SAE permission of **Start Run**.

In order to save the template file, your SAE user account must have the SAE permission of **Save Pre Run**.

Add a qPCR instrument (see “Related documentation” on page 31).

1. Enter the name of the qPCR run in the **Run name** field.
   The default run name is the assay definition file name appended with the date and time.
2. *(Optional)* Enter the plate barcode in the **Plate barcode** field.
   - Enter the barcode number manually.
   - Use the barcode scanner to scan the plate barcode.
3. *(Optional)* Enter a comment in the **Plate comment** field.
4. *(Optional)* Click **Show run method** to view the run method, then click **Hide run method**.

5. Save or send the qPCR run.

| Destination | Option |
|---|---|
| No destination selected | Click **Save for later**. |
| Instrument | Click **Save for later**, or click **Save & start run**.<br>If you click **Save & start run**, the **Confirm run & destination** dialog box is displayed. Ensure that the plate is loaded into the instrument and that the instrument drawer is closed. Click **Start run**. |

   The options to send the run to the instrument depend on what the instrument supports.

6. Complete the e-signature requirements, if required.

   This step is required only if the SAE Administrator Console Dx was set up to require e-signatures in order to start a run.

The run is added to the **qPCR** tab.

## Start the real-time PCR run using Diomni™ Software v4.0 or later (RUO workspace only)

1. Return to Diomni™ Software v4.0 or later (RUO workspace only).
2. In the **RUO** workspace, select the **qPCR** tab.
3. Find the recently created real-time PCR run **ID**.
4. Click the run **ID** to open the plate map screen.
5. Verify the following run details:

   **Note:** Confirm target information in the ADF (see “Import and edit the assay definition file” on page 9).

**Table 8 QuantStudio™ instrument thermal protocol**

| Step | Temperature | Time | Number of cycles | Ramp rate |
|---|---|---|---|---|
| UNG incubation | 25°C | 2 minutes | 1 | 1.6°C per second |
| Preincubation | 53°C | 10 minutes | 1 | 1.6°C per second |
| | 85°C | 10 minutes | 1 | 1.6°C per second |
| Activation | 95°C | 2 minutes | 1 | 1.6°C per second |
| Denaturation | 95°C | 3 seconds | 43 | 1.6°C per second |
| Anneal / extension | 60°C | 32 seconds | | 1.6°C per second |

**Table 9 PCR filters for the QuantStudio™ instruments using the TrueMark™ SARS-CoV-2, Flu A, Flu B, RSV Select Panel**

| | | Emission Filter | | | | | |
|---|---|---|---|---|---|---|---|
| | | m1(520±15) | m2(558±11) | m3(586±10) | m4(623±14) | m5(682±14) | m6(711±12) |
| Excitation Filter | x1(470±15) | ✔ | ✔ | ✔ | | | |
| | x2(520±10) | | ✔ | ✔ | ✔ | | |
| | x3(550±11) | | | ✔ | ✔ | | |
| | x4(580±10) | | | | ✔ | ✔ | |
| | x5(640±10) | | | | | ✔ | |
| | x6(662±10) | | | | | | |

6. Click **Next** to advance to the **Save plate setup** step, then select the applicable instrument.
7. Select **Save & start run**.
8. Enter **Audit reason** if applicable.
9. Click **Save & start run**.
10. Load the plate and place a MicroAmp™ Optical Film Compression Pad with the gray side down on the surface of the real-time PCR reaction plate. Ensure there is a proper seal between the thermal cycler and the adhesive film.

    **IMPORTANT!** Ensure that the PCR plate is in the instrument before clicking **Start run**. Once the run is sent to the instrument, the run will start automatically.
11. Click **Start run**.
12. In the **qPCR** tab, verify the PCR run status is set to **Running**.

    **Note:** Once the run completes, the run is automatically removed from the **qPCR** tab and is added to the **Results** tab.
13. When the run is complete, touch ⏏ **(Eject)** on the instrument touch screen to open the real-time PCR instrument plate tray and dispose of or store the real-time PCR plate according to your laboratory procedures.

    **Note:** If the instrument is in sleep mode, touch the screen. If prompted, sign in with your username and password/PIN.

14. At the end of the run, remove the MicroAmp™ Optical Film Compression Pad from the plate and store the compression pad inside the pack.

**IMPORTANT!**

- If the compression pad becomes stuck inside the thermal cycler, call service to clean the heated cover.
- Between each use, place the pad back in the pouch so that it does not dry out.
- Each compression pad may be used up to 20 times before discarding. Do not use more than 20 times.
- Do NOT use the pad with other instruments, unless expressly instructed to do so in the user documentation.

# Analyze the data

Use either Diomni™ Software v4.0 or later (RUO workspace only) or QuantStudio™ Design and Analysis v2.7 or later for data analysis.

For QuantStudio™ Design and Analysis v2.7, proceed to step 1.

For Diomni™ Software v4.0 or later (RUO workspace only), proceed to step 2.

**Note:** For detailed information about data analysis, see the appropriate documentation for your instrument.

1. For QuantStudio™ Design and Analysis v2.7, perform the following:
   a. In the QuantStudio™ Design and Analysis v2.7 or later home screen, open the data file (EDS).
   b. In the open data file, click **Actions** ▸ **Save As**, then save the data file with a new name.

      **Note:** QuantStudio™ Design and Analysis v2.7 or later requires data files created on the QuantStudio™ 5 Real-Time PCR System to be saved as a new data file.

   c. In the analysis settings, select automatic baseline with a start cycle of 5 and an end cycle of auto, or select according to lab validation.
   d. Set the appropriate threshold values for each target, as established by your laboratory.

      **IMPORTANT!** Do not use automatic threshold values.

   e. Determine $C_q$ cutoff values for each target for samples and controls.

      **Note:** QuantStudio™ Design and Analysis v2.7 or later reports $C_q$ values instead of $C_t$ values. The $C_q$ values are equivalent to $C_t$ values.

   f. In the **Primary Analysis Setting** window, click on the **Advanced** tab.
   g. In the **Auto Spectral Conditioning** settings, click **Update**.
   h. Once the open file dialog box is open, locate and select the `asa-v1.4-r5.cfg` file.

i. Select the **Reduce dye signal crosstalk by algorithm (it may slow down your analysis)** checkbox.

---

**Note:** The ASA algorithm decreases spectral crosstalk. This reduction enables lower thresholds and reduces the potential for false positives and negatives.

For more information on using the `asa-v1.4-r5.cfg` file in an assay template (EDT) file, contact your local support team.

---

2. For Diomni™ Software v4.0 or later (RUO workspace only), perform the following:
   a. In the RUO workspace, select the **Results** tab.
   b. Click the run name to open the post-run file, then select either the **Summary** tab to view the summary of the run or the **Additional Information** tab to view additional run information.
   c. For additional results analysis, see “Related documentation” on page 31.
3. Analyze results according to analysis, interpretation, and QC parameters, as established by your laboratory.

# A Safety



**WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

# Chemical safety

**WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Biohazardous materials may require special handling, and disposal limitations may apply.

# Biological hazard safety

**WARNING! Potential Biohazard.** Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  **www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf**
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  **www.who.int/publications/i/item/9789240011311**

B

# Documentation and support

## Related documentation

| Document | Publication Number |
|---|---|
| *QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide* | MAN0010407 |
| *QuantStudio™ 5 Dx Real-Time PCR Instrument Maintenance and Administration User Guide* (RUO mode only) | 100091230 |
| *QuantStudio™ Real-Time PCR Software Getting Started Guide* | 4489822 |
| *QuantStudio™ Design and Analysis Software v2 User Guide* | MAN0018200 |
| *QuantStudio™ Design and Analysis Desktop Software User Guide* | MAN0010408 |
| Diomni™ Software v4.0 User Guide (RUO workflow only) | 100119902 |
| *Diomni™ Software v4.0 Assay Definition User Guide* (RUO workflow only) | 100119903 |
| *SAE Administrator Console Dx v1.2 User Guide (for use with the Diomni™ Software v4.0)* (RUO workflow only) | 100119904 |
| *MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit User Guide* | MAN0024756 |
| AcroMetrix™ Multi-Analyte SARS-CoV-2, Flu A/B, RSV A/B Control Package Insert | 954516 |

## Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support

- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  **Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

# Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.