TaqMan™ SARS-CoV-2, Flu A/B, RSV Multiplex Assay

Pub. No. MAN0019614 Rev. C.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The TaqMan™ SARS-CoV-2, Flu A/B, RSV Multiplex Assay is a multiplex real-time RT-PCR assay for the detection of RNA from the SARS-CoV-2 virus, influenza A and B viruses, and respiratory syncytial virus (RSV) subtypes A and B.

The assay requires the following components:

- TaqMan™ SARS-CoV-2, Flu A/B, RSV RT-PCR Assay Kit, includes the following components:
  - TaqMan™ SARS-CoV-2, Flu A/B, RSV Multiplex Assay—Multiplexed assays that contain primer and probe sets specific to the following targets (see Table 1 on page 1):
    - SARS-CoV-2 N gene and SARS-CoV-2 S gene
    - Flu A/B
    - RSV
    - MS2
  - TaqMan™ MS2 Phage Control—Internal process control for nucleic acid extraction
- TaqMan™ SARS-CoV-2, Flu A/B, RSV RNA Control—RNA control that contains targets specific to the SARS-CoV-2, influenza A and B, and RSV genomic regions targeted by the assays
- TaqMan™ Control Dilution Buffer—Dilution buffer for the control
- TaqPath™ 1-Step Multiplex Master Mix

Table 1 Dyes, quenchers, and targets

Dye	Quencher	Target
FAM™ dye	QSY™ quencher	Flu (A and B)
VIC™ dye	QSY™ quencher	SARS-CoV-2 (N gene and S gene)
ABY™ dye	QSY™ quencher	RSV
JUN™ dye	QSY™ quencher	MS2

Note: The viral targets are each in a single optical channel and cannot be differentiated.

For catalog numbers and storage conditions, see “Contents and storage” on page 2.

IMPORTANT! It is the responsibility of the laboratories using the TaqMan™ SARS-CoV-2, Flu A/B, RSV Multiplex Assay to design and validate their own experimental design and analysis parameters.

Contents and storage

The items listed in the following table are required for the TaqMan™ SARS-CoV-2, Flu A/B, RSV Multiplex Assay. The items listed are sufficient for 1,000 reactions.

Kit or product	Cat. No.	Components	Amount	Storage
TaqMan™ SARS-CoV-2, Flu A/B, RSV RT-PCR Assay Kit	A47702	TaqMan™ SARS-CoV-2, Flu A/B, RSV Multiplex Assay	1,500 µL	–30°C to –10°C
		TaqMan™ MS2 Phage Control	11 × 1 mL	–30°C to –10°C
TaqMan™ SARS-CoV-2, Flu A/B, RSV RNA Control	956126	—	10 × 10 µL	≤ –70°C
TaqMan™ Control Dilution Buffer	A49889	—	10 × 250 µL	–30°C to –10°C
TaqPath™ 1-Step Multiplex Master Mix	A28523	—	10 mL	–30°C to –10°C

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.

IMPORTANT! The customer is responsible for performing all of the necessary validations to run this assay.

Item	Source
Real-time PCR instrument	
An Applied Biosystems™ real-time PCR instrument compatible with the four dyes listed in Table 1 on page 1. The assay has been tested with the following instrument: Applied Biosystems™ 7500 Fast Real-Time PCR Instrument (used with SDS Software v1.5.1 or 7500 Software v2.3)	Contact your local sales office
Software	
QuantStudio™ Design and Analysis Software v2.4.3 or later	thermofisher.com/us/en/home/global/formas/life-science/quantstudio-6-7-pro-software
Equipment	
Laboratory freezers • –30°C to –10°C • ≤ –70°C	MLS
Centrifuge, with a rotor that accommodates standard and deepwell microplates	MLS
Microcentrifuge	MLS
Laboratory mixer, vortex or equivalent	MLS
Single and multichannel adjustable pipettors (1.00 µL to 1,000.0 µL)	MLS
Cold block (96-well or 384-well) or ice	MLS

Item	Source
Automated nucleic acid extraction system and materials	
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: • 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	 • 97002540 • 267600 • 167008 • 269787 • AB0796 • AB1127 • 137101 • 136101 • 95040450, A48305, A48424, 95040455
KingFisher™ 96 tip comb for DW magnets	97002534, A48438, A48414
Kits and reagents	
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit	A48383R
Fisher BioReagents™ Ethanol, Absolute, Molecular Biology Grade[1], or equivalent	BP2818100, BP2818500, BP28184
Nuclease-free Water (not DEPC-Treated)	MLS
Calibration plates (7500 Real-Time PCR Instrument series)	
ABY™ Dye Spectral Calibration Plate for Multiplex qPCR, Fast 96-well (0.1-mL)	A24734
JUN™ Dye Spectral Calibration Plate for Multiplex qPCR, Fast 96-well (0.1-mL)	A24735
Tubes, plates, and other consumables	
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	4346906, 4366932
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL	4346907
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Adhesive Film	4311971, 4360954
MicroAmp™ Adhesive Film Applicator	4333183
Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)	thermofisher.com/plastics
Sterile aerosol barrier (filtered) pipette tips	thermofisher.com/pipettetips

[1] Available at fisherscientific.com.

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 10% bleach or decontamination solution, followed by 70% ethanol
  - Use of ultraviolet light during biosafety cabinet decontamination (when available)
- To prevent degradation, keep eluted sample RNA, master mixes, assays, and controls on ice or in cold blocks while in use. Limit freeze-thaw cycles.
- Aliquot reagents to prevent stock contamination and reduce the number of freeze-thaw cycles.
- After each run, review the amplification curves in the instrument software according to data QC standard operating procedures for your lab.

Extract RNA

IMPORTANT! It is the responsibility of the laboratories to validate their own experimental design, including RNA extraction.

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 400 µL of sample.
- Determine the number of required reactions based on the number of samples, plus one Negative Control per plate.
- Prepare fresh 80% Ethanol using Ethanol, Absolute, Molecular Biology Grade and Nuclease-free Water (not DEPC-Treated) 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

- Mark the Negative Control well on the 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_400_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	1,000 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 DW 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 Total Nucleic Acid Magnetic 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]	Volume per 96-well plate
Binding Solution	530 µL	56.0 mL
Total Nucleic Acid Magnetic Beads	20 µL	2.1 mL
Total volume per well	**550 µL**	**58.1 mL**

[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 a Proteinase K and TaqMan™ MS2 Phage Control Mix

Prepare the required amount of the Proteinase K and TaqMan™ MS2 Phage Control Mix on each day of use. Keep on ice.

1. Thaw the vial of TaqMan™ MS2 Phage Control.
2. For the number of required reactions, prepare the Proteinase K and TaqMan™ MS2 Phage Control Mix according to the following table:

Component	Volume per well[1]	Volume per 96-well plate
Proteinase K	10 µL	1,056 µL
TaqMan™ MS2 Phage Control	10 µL	1,056 µL
Total volume per well	**20 µL**	**2,112 µL**

[1] Include 10% overage when preparing the Proteinase K and TaqMan™ MS2 Phage Control Mix for use with multiple reactions.

3. Mix well by inversion, then store on ice.

Prepare sample plate

Prepare the Proteinase K and TaqMan™ MS2 Phage Control Mix (see "Prepare a Proteinase K and TaqMan™ MS2 Phage Control Mix" on page 6).

1. Invert the Binding Bead Mix 5 times gently to mix, then add 550 µL to each sample well and the Negative Control well in the Sample 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 400 µL of sample to each sample well.
3. Add 400 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
4. Add 20 µL of the Proteinase K and TaqMan™ MS2 Phage Control Mix to each well in the KingFisher™ 96 Deep-Well Plate labeled "Sample Plate", including the Negative Control well.

Process the samples

1. Select the **MVP_2Wash_400_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 (~24 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 5).

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 RT-PCR reactions

Guidelines for RT-PCR

IMPORTANT!

- Prepare the run plate on ice and keep it on ice until it is loaded into the real-time PCR instrument.
- Run the plate immediately after preparation. Failure to do so could result in degraded RNA samples.
- To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Do not use the same pipette for controls and RNA samples, and always use aerosol barrier pipette tips.
- Maintain an RNase-free environment.
- Protect assays from light.
- Keep RNA samples and components on ice during use.
- For each RT-PCR plate, include the following controls:
  - One Positive Control
  - One Negative Control from each extraction run.

Prepare the RT-PCR reactions

1. If frozen, thaw the reagents on ice.
2. Gently vortex the reagents, then centrifuge briefly to collect liquid at the bottom of the tube.
3. Dilute TaqMan™ SARS-CoV-2, Flu A/B, RSV RNA Control to a working stock (1/3,500 dilution):
   a. Pipet 98.0 µL of TaqMan™ Control Dilution Buffer into a microcentrifuge tube, then add 2.0 µL of TaqMan™ SARS-CoV-2, Flu A/B, RSV RNA Control. Mix well, then centrifuge briefly.
   b. Pipet 138.0 µL of TaqMan™ Control Dilution Buffer into a second microcentrifuge tube, then add 2.0 µL of the dilution created in substep 3a. Mix well, then centrifuge briefly.

   Note: The TaqMan™ SARS-CoV-2, Flu A/B, RSV RNA Control does not contain the MS2 template.
4. Prepare the Reaction Mix:
   a. For each run, combine the following components sufficient for the number of RNA samples plus one Positive Control and one Negative Control.

   All volumes include 10% overage for pipette error.

Component	Volume per RNA Sample or Control	Volume for *n* RNA Samples plus 2 Controls	Volume for 94 RNA Samples plus 2 Controls
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	6.25 µL	6.875 x (*n* + 2) µL	660 µL
TaqMan™ SARS-CoV-2, Flu A/B, RSV Multiplex Assay	1.25 µL	1.375 x (*n* + 2) µL	132 µL
Total Reaction Mix volume	**7.5 µL**	**—**	**792 µL**

5. Set up the reaction plate:
   a. Pipette 7.5 µL of the Reaction Mix prepared in step 4 into each well of a MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL.

      Plates without a barcode can be used (see “Required materials not supplied” on page 2).
   b. Gently vortex the sealed plate containing the purified sample RNA and Negative Control from the RNA extraction procedure, then centrifuge briefly to collect liquid at the bottom of the plate.
   c. Unseal the plate containing the purified sample RNA and Negative Control from the RNA extraction procedure. Add either sample RNA, Negative Control, or Positive Control to each well of the reaction plate according to Table 2 on page 8.
   d. Seal the plate thoroughly with MicroAmp™ Optical Adhesive Film.

   IMPORTANT! 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 PCR.

e. Vortex the plate at the highest setting speed for 10–30 seconds with medium pressure. Move the plate around to ensure equal contact on the vortex mixer platform.

IMPORTANT! Vortex for 10–30 seconds to ensure proper mixing. Failure to do so might result in inaccurate sample results.

f. Centrifuge the reaction plate for 1–2 minutes at ≥650 × *g* (≥650 RCF) to remove bubbles and to collect the liquid at the bottom of the reaction plate.

Table 2 Reaction plate

Component	Volume per reaction		
	RNA Sample reaction	Positive Control reaction	Negative Control reaction
Reaction Mix (from step 4)	7.5 µL	7.5 µL	7.5 µL
Purified sample RNA (from RNA extraction)	17.5 µL	—	—
Positive Control (diluted TaqMan™ SARS-CoV-2, Flu A/B, RSV RNA Control from step 3)	—	17.5 µL	—
Negative Control (from RNA extraction)	—	—	17.5 µL
Total volume	**25.0 µL**	**25.0 µL**	**25.0 µL**

Set up and run the real-time PCR instrument

Calibration

Ensure that your real-time PCR instrument is calibrated for the dyes listed in Table 1 on page 1. See your instrument user guide for more information.

Dye calibration for the 7500 Real-Time PCR Instrument series

A maintained instrument will be calibrated for many dyes, including FAM™ dye and VIC™ dye. In addition to those dyes, the instrument operator must calibrate the instrument for ABY™ dye and JUN™ dye that are used with this kit. For all other assays, refer to the standard calibration process.

Perform RT-PCR

For more information about the instrument, see “Related documentation” on page 9.

1. Set up and run the real-time PCR instrument with the following settings.
   - Assay: Standard curve
   - Run mode: Standard
   - Passive reference set to **None**
   - Sample volume: 25 µL

2. Set up the following reporter dye and detector pairs.

Reporter dye	Detector
FAM	Flu (A and B)
VIC	SARS-CoV-2 (N and S protein)
ABY	RSV
JUN	MS2

3. Set up the thermal protocol.

Step	Temperature	Time	Number of cycles
UNG incubation	25°C	2 minutes	1
Reverse transcription	53°C	10 minutes	1
Preincubation	85°C	10 minutes	1
Activation	95°C	2 minutes	1
Denaturation	95°C	3 seconds	46
Anneal / extension	60°C	30 seconds	

4. Load the plate and start the instrument run.

Analyze data

IMPORTANT! It is the responsibility of the laboratories using the TaqMan™ SARS-CoV-2, Flu A/B, RSV Multiplex Assay to design and validate their own experimental design and analysis parameters.

For more information about using the software, see "Related documentation" on page 9.

Note: QuantStudio™ Design and Analysis Software v2 reports C_q values instead C_t values. The C_q values are equivalent to the C_t values indicated for data analysis and interpretation.

Use QuantStudio™ Design and Analysis Software v2.4.3 or later.

1. Open the data file (SDS) in the data analysis software.

   Note: QuantStudio™ Design and Analysis Software v2 requires data files created on a 7500 Fast Real-Time PCR Instrument to be saved as a new data file. Click **Actions ▸ Save As**, then save the data file with a new name.

2. Use automatic baselining with a start cycle of 5.

3. Set the appropriate threshold values for each target, as validated by your laboratory.

   IMPORTANT! Do not use automatic threshold values.

4. Determine C_t/C_q cutoff values for each target for samples and controls.

5. Analyze results according to analysis, interpretation, and QC parameters, as validated by your laboratory.

   IMPORTANT! After each run, review the data in the QuantStudio™ Design and Analysis Software v2.4.3 or later with the **Linear Plot** view. Zoom the y-axis to **-10,000 to 50,000**. This can identify amplification curves with inconsistencies that lead to inaccurate results.

Contact Support for more information.

Related documentation

Document	Publication Number
Applied Biosystems™ 7500/7500 Fast Real-Time PCR System: Maintenance Guide	4387777
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (automated extraction) User Guide	MAN0018073
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit User Guide	MAN0024756
Thermo Scientific™ KingFisher™ Flex User Manual	N07669
QuantStudio™ Design and Analysis Software v2 User Guide	MAN0018200

Limited product warranty

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Revision	Date	Description
C.0	22 September 2022	The amount of MS2 Phage Control was increased to 11 (“Contents and storage” on page 2).
B.0	28 April 2021	• Removed early access label. • Updated RNA extraction procedure for bead carry over. • Added information about Binding Bead Mix incompatibility with bleach. • Updated catalog number for MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit and added user guide to related documentation. • Updated link for QuantStudio™ Design and Analysis Software v2.
A.0	15 October 2020	New document.

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