Respiratory Tract Microbiota Profiling Experiments v2

APPLICATION GUIDE

TaqMan™ Assays for respiratory tract microbiota profiling experiments in TaqMan™ Array Card format

for use with:
TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card
Custom TaqMan™ Array Cards
MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit
QuantStudio™ 12K Flex Real–Time PCR System
QuantStudio™ 7 Flex Real-Time PCR System

Catalog Number A49047
Publication Number MAN0019507
Revision C.0

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ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision history: Pub. No. MAN0019507

Revision	Date	Description
C.0	12 February 2021	Updated centrifuges.
B.0	5 January 2021	Updated product information, disclaimer, and related documentation. Added Custom TaqMan™ Array Cards.
A.0	29 October 2020	New document.

Contents

1 Overview

Product description

TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card (Cat. No. A49047) is an efficient, easy-to-use TaqMan™ Array Card for the characterization of key respiratory tract microbes. The array card includes 44 TaqMan™ assays that have been optimized for detection of 41 respiratory tract viral and bacterial, and fungal nucleic acid, including two assays that target SARS-CoV-2. The array card also includes control assays for TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*), TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control, the human RNase P RPPH1 gene, and the human 18S ribosomal RNA gene. For a complete list of assays included in the array card, see "TaqMan™ assays included in the TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card" on page 5.

The assays perform well with total nucleic acid that is isolated from nasopharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage (BAL) research samples using the MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit.

TaqMan™ assay designs and assay target sequences have undergone rigorous bioinformatics selection and analysis to maximize strain coverage and minimize potential for off-target cross-reactivity. Qualified TaqMan™ assays also undergo performance testing to ensure that results are accurate with high levels of sensitivity and specificity.

TaqMan™ assays for respiratory tract microbiota profiling

TaqMan™ assays included in the TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card

The following assays are included in the TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card.

The assays can also be ordered in a Custom TaqMan™ Array Card.

Table 1 Assays for respiratory tract microbiota targets

Target organism	Assay name	Nucleic acid type	Assay ID
Bacteria			
Bordetella bronchiseptica / parapertussis / pertussis	Bordetella	DNA	Ba06439624_s1
Bordetella pertussis	B.pertussis	DNA	Ba06439623_s1
Chlamydophila pneumoniae	C.pneumoniae	DNA	Ba06439616_s1
Haemophilus influenzae	H.influenzae	DNA	Ba06439625_s1

Table 1 Assays for respiratory tract microbiota targets *(continued)*

Target organism	Assay name	Nucleic acid type	Assay ID
Klebsiella pneumoniae complex	K.pneumoniae	DNA	Ba04932083_s1
Legionella pneumophila	L.pneumophila	DNA	Ba06439617_s1
Moraxella catarrhalis	M.catarrhalis	DNA	Ba06439622_s1
Mycoplasma pneumoniae	M.pneumoniae	DNA	Ba06439620_s1
Staphylococcus aureus	S.aureus	DNA	Ba04646259_s1
Streptococcus pneumoniae	S.pneumoniae	DNA	Ba06439619_s1
Fungus			
Pneumocystis jirovecii	P.jirovecii	DNA	Fn06439626_s1
Virus			
Adenovirus	AdV_1of2	DNA	Vi99990001_po
Adenovirus	AdV_2of2	DNA	Vi99990002_po
Human Bocavirus	HBoV	DNA	Vi99990003_po
Human Coronavirus 229E	CoV_229E	RNA	Vi06439671_s1
Human Coronavirus HKU1	CoV_HKU1	RNA	Vi06439674_s1
Human Coronavirus NL63	CoV_NL63	RNA	Vi06439673_s1
Human Coronavirus OC43	CoV_OC43	RNA	Vi06439646_s1
Human Enterovirus (pan assay)	EV_pan	RNA	Vi06439631_s1
Human Enterovirus D68	EV_D68	RNA	Vi06439669_s1
Human Metapneumovirus (hMPV)	hMPV	RNA	Vi99990004_po
Human Parainfluenza virus 1	hPIV1	RNA	Vi06439642_s1
Human Parainfluenza virus 2	hPIV2	RNA	Vi06439672_s1
Human Parainfluenza virus 3	hPIV3	RNA	Vi06439670_s1
Human Parainfluenza virus 4	hPIV4	RNA	Vi99990005_po
Human Parechovirus	HPeV	RNA	Vi99990006_po
Human Respiratory Syncytial Virus A (RSVA)	RSVA	RNA	Vi99990014_po
Human Respiratory Syncytial Virus B (RSVB)	RSVB	RNA	Vi99990015_po
Human Rhinovirus 1/2	RV_1of2	RNA	Vi99990016_po
Human Rhinovirus 2/2	RV_2of2	RNA	Vi99990017_po

Table 1 Assays for respiratory tract microbiota targets *(continued)*

Target organism	Assay name	Nucleic acid type	Assay ID
Human herpesvirus 3 (HHV3 – Varicella zoster Virus)	HHV3	DNA	Vi06439647_s1
Human herpesvirus 4 (HHV4 – Epstein-Barr Virus)	HHV4	DNA	Vi06439675_s1
Human herpesvirus 5 (HHV5 – Cytomegalovirus)	HHV5	DNA	Vi06439643_s1
Human herpesvirus 6 (HHV6)	HHV6	DNA	Vi06439627_s1
Influenza A	Flu_A_pan	RNA	Vi99990011_po
Influenza A/H1-2009	Flu_A_H1	RNA	Vi99990009_po
Influenza A/H3	Flu_A_H3	RNA	Vi99990010_po
Influenza B	Flu_B_pan	RNA	Vi99990012_po
Measles virus	Measles	RNA	Vi99990013_po
Middle East Respiratory Syndrome coronavirus (MERS-CoV)	MERS_CoV	RNA	Vi06439644_s1
Mumps virus	Mumps	RNA	Vi06439657_s1
Severe Acute Respiratory Syndrome coronavirus (SARS-CoV)	SARS_CoV	RNA	Vi06439634_s1
Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2); S protein gene	SARS-CoV2S	RNA	Vi07918636_s1
Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2); N protein gene	SARS-CoV2N	RNA	Vi07918637_s1

Table 2 Assays for respiratory tract microbiota controls

Control name	Assay name	Nucleic acid type	Assay ID
TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*)	B.atrophaeus	DNA	Ba06596576_s1
TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control	Xeno	RNA	Ac00010014_a1
Human RNase P RPPH1 gene	RPPH1	DNA	Hs04930436_g1
Human 18S ribosomal RNA gene[1]	18S	DNA	Hs99999901_s1

[1] Human 18S ribosomal RNA is used as a manufacturing control for the array card and is not an assay specific to Respiratory Tract Microbiota profiling.

Additional TaqMan™ assays for respiratory tract microbiota profiling

The following TaqMan™ assays are not included in the TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card. For respiratory tract microbiota profiling using these assays, use a Custom TaqMan™ Array Card.

Target organism	Assay name	Nucleic acid type	Assay ID
Bacteria			
Bordetella holmesii	B.holmesii	DNA	Ba06439621_s1
Coxiella burnetii	C.burnetii	DNA	Ba06439618_s1

TaqMan™ Array Card products and formats

TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card

The TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card (Cat. No. A49047) contains pre-plated, dried down TaqMan™ assays for respiratory tract microbiota profiling. For the complete lists of assays included with the array card, see "TaqMan™ assays included in the TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card" on page 5.

Contents and storage

Table 3 TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card (Cat. No. A49047; format 48 assays and up to 8 samples)

Component	Amount	Storage
TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card	1 card	2–8°C

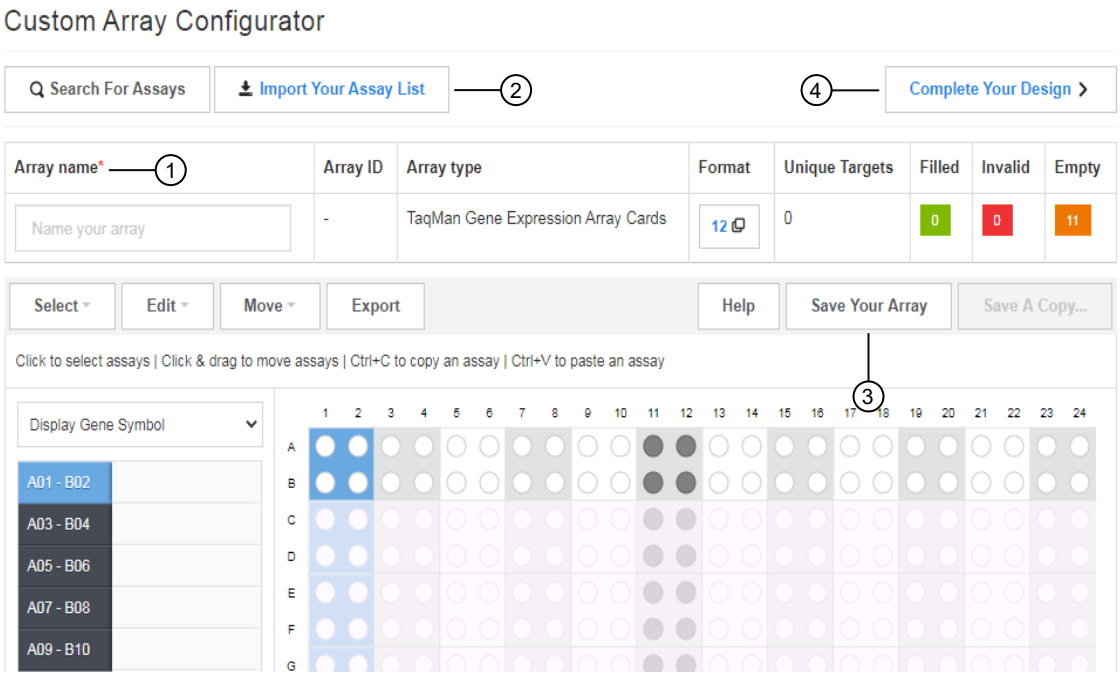
Custom TaqMan™ Array Card formats

Any of the TaqMan™ assays for respiratory tract microbiota profiling can be included in a Custom TaqMan™ Array Card.

Array format	Manufacturing controls	Number of assays/replicated	Maximum number of samples
24	1	23/2	8
48	1	47/1	8

Configure and order Custom TaqMan™ Array Cards

1. Go to **thermofisher.com/order/custom-array**.
2. For array type, select **TaqMan™ Gene Expression Array Cards**.
3. *(Optional)* In the table, click **View Layout** to preview the layout of the card.
4. In the table, click **Select** to configure a card with the desired array format.
   The **Custom Array Configurator** screen displays.



① **Array name**
② **Import Your Assay List**
③ **Save Your Array**
④ **Complete Your Design**

5. Enter the custom array name in the **Array name** text field.
6. Click **Import Your Assay List**, then upload or copy-paste the assay information:
   - Under **Upload a list of Assay IDs**, click **Choose File**, then select a tab-delimited text file (TXT) containing Assay IDs.

     or
   - Under **Enter a list of Assay IDs**, paste the Assay IDs, then click **Import Entered List**.
7. Follow the on-screen instructions to configure the assays on the card.

8. *(Optional)* Click **Save Your Array** at any time to save the array configuration to your Thermo Fisher Scientific account.

9. When the plate is configured, click **Complete Your Design**, then follow the on-screen instructions to complete the order.

Materials required but 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.

Materials required for nucleic acid isolation

Nucleic acid isolation kit

Table 4 MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit (Cat. No. A42356)

Component	Amount	Storage
Binding Solution	60 mL	15–25°C
Wash Solution	100 mL	
Elution Solution	10 mL	
Nucleic Acid Binding Beads	2 mL	
Proteinase K	1 mL	
Enzyme Mix	5 mL	–25°C to –15°C

Additional materials

Item	Source
Instrument and equipment	
KingFisher™ Flex Magnetic Particle Processor 96DW with deep-well heat block	5400630
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Plastics and consumables	
KingFisher™ 96 Deep-Well Plate	95040450
KingFisher™ 96 KF microplate (200 µL)	97002540
KingFisher™ 96 tip comb for DW magnets	97002534

(continued)

Item	Source
Conical Tubes (15 mL)	AM12500
Conical Tubes (50 mL)	AM12501
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	AM12450
Nonstick, RNase-Free Microfuge Tubes, 2.0 mL	AM12475
MicroAmp™ Clear Adhesive Film	4306311
Filtered micropipettor tips	MLS
Reagent reservoirs	MLS
Reagents	
Ethanol, 100% (molecular biology grade)	MLS
Nuclease-free Water	AM9932, or equivalent
Universal Transport Media, for preparation of negative extraction control	Fisher Scientific 22-031-14, or equivalent
(*Optional*) 1X PBS (1X), pH 7.4, for reconstitution of TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*)	10010023

Materials required for PCR

Table 5 Materials required for preamplification and real-time PCR

Item	Source
Equipment	
Microcentrifuge	MLS
Vortex mixer	MLS
Micropipettes	MLS
Tubes, plates, and other consumables	
MicroAmp™ Optical 96-Well Reaction Plate	N8010560, or equivalent; see **thermofisher.com/plastics**
MicroAmp™ Clear Adhesive Film	4306311
Aerosol-resistant barrier pipette tips	MLS
Disposable gloves	MLS
Reagents	
Nuclease-free Water	AM9937, or equivalent

Table 6 Additional materials required for preamplification

Item	Source
Thermal cycler, one of the following (or equivalent): • Veriti™ Thermal Cycler, 96-well standard block • SimpliAmp™ Thermal Cycler • ProFlex™ PCR System	Contact your local sales office
TaqPath™ 1-Step RT-qPCR Master Mix, CG	• A15299 (5 x 1 mL) • A15300 (1 x 10 mL)
TrueMark™ Respiratory Panel 2.0 PreAmp Primers	A49049

Table 7 Additional materials required for real-time PCR with TaqMan™ Array Cards

Item	Source
Real-time PCR instrument, one of the following; configured with the TaqMan™ Array Card block and heated cover.	
QuantStudio™ 7 Flex Real-Time PCR System	Contact your local sales office
QuantStudio™ 12K Flex Real–Time PCR System	
Equipment	
TaqMan™ Array Card Sealer	Contact your local sales office
Centrifuge with custom buckets and card holders, one of the following: • Sorvall™ centrifuge • Multifuge™ centrifuge • Megafuge™ centrifuge See the **Resources** section at **thermofisher.com/taqmanarrays** for a list of compatible centrifuges, rotors, and buckets.	Contact your local sales office
Blank balance TaqMan™ Array Cards (Included with the instrument block upgrade / installation kit)	Contact your local sales office
Reagents	
TaqMan™ Fast Advanced Master Mix, No UNG	• A44359 (1 mL) • A44360 (5 mL)

Materials required for data analysis

Item	Source
Software, select one of the following:	
Relative Quantification Application (recommended)	apps.thermofisher.com
QuantStudio™ 12K Flex Software	Included with QuantStudio™ 12K Flex Real–Time PCR System
QuantStudio™ Real-Time PCR Software	Included with QuantStudio™ 6 and 7 Flex Real-Time PCR System

Optional controls

Control	Purpose	How to use	Cat. No.
TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control	Synthetic RNA control for RNA recovery, reverse transcription, preamplification, and PCR	Nucleic acid isolation: Add to samples along with the Binding/Bead Mix	A39179
TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*)	Lyophilized organism control for nucleic acid extraction and purification	Nucleic acid isolation: Stand-alone sample, or add to samples after Enzyme Mix	A39180
TrueMark™ Respiratory Panel 2.0 Amplification Control	DNA plasmid control for real-time PCR	Real-time PCR: Stand-alone sample added directly to the card	A48101

TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control

TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control is a synthetic RNA control that serves as an exogenous process control for nucleic acid isolation and RNA recovery, reverse transcription, preamplification, and PCR. The control is used with the proprietary TaqMan™ assay for Xeno™ sequences, which is included in the TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card.

TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control is supplied at a concentration of 10,000 copies/µL. During nucleic acid isolation, 10 µL of the control can be added to each test sample along with the nucleic acid binding reagents (Binding Solution). When carried through the respiratory tract microbiota workflow, the control is used to monitor nucleic acid recovery, RNA reverse transcription, cDNA preamplification, and PCR. The control can be used to identify sample-specific amplification inhibition, which reduces the likelihood of false negatives and provides confidence that results are accurate. It is recommended that the control is added to each sample during nucleic acid isolation.

TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*)

TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*), serves as a process control for cell lysis and nucleic acid recovery. The control is used with the proprietary TaqMan™ assay for *Bacillus atrophaeus* sequences.

Like other gram-positive bacteria, *Bacillus atrophaeus* has thick cell walls than can be difficult to lyse. This characteristic makes gram-positive bacteria an ideal control to monitor the efficiency of cell lysis and subsequent nucleic acid recovery.

TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*) is supplied lyophilized with a quantity of 1×10^9 copies/vial, and is reconstituted in 200 µL of 1X PBS (1X), pH 7.4 to a final concentration 5×10^6 copies/µL. During nucleic acid isolation, 10 µL of the control is processed as a stand-alone sample in a background of universal transport media. It can be added to the negative extraction control, and may also be added to one or more test samples at the start of the extraction process. The control is carried through the remainder of the workflow with the test samples. It is recommended that at least one stand-alone control sample is run per extraction plate.

TrueMark™ Respiratory Panel 2.0 Amplification Control

TrueMark™ Respiratory Panel 2.0 Amplification Control contains a linearized multi-target plasmid with target sequences for each respiratory tract microbiota assay on the TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card. The plasmid also contains target sequences for TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control, TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*), and the human RNase P RPPH1 gene. It can be included in respiratory tract microbiota profiling experiments as a positive control for panel-specific amplification.

TrueMark™ Respiratory Panel 2.0 Amplification Control is supplied at a concentration of 1×10^5 copies/µL. During real-time PCR, 10 µL of the control is used as a stand-alone sample in one port of the TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card. The control can be used if needed to verify assay performance and to help with troubleshooting.

Note: The amplification control RV target sequence is a perfect match to the RV_1of2 assay target, and contains a mismatch with the RV_2of2 assay target. Lower Amp Scores and C_q confidence scores can be noted for RV_2of2 versus RV_1of2.

Workflow using TaqMan™ Array Cards

Start with bronchoalveolar lavage, nasopharyngeal swab, or nasopharyngeal aspirate samples

▼

Isolate nucleic acid using MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit (page 16)

▼

Perform preamplification (page 20)

▼

Prepare and run TaqMan™ Array Cards (page 23)

▼

Analyze data (page 25)

2 Isolate nucleic acid using MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit

For required materials, see “Materials required for nucleic acid isolation” on page 10.

Procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Ensure that Nucleic Acid Binding Beads remain in a homogeneous suspension while pipetting. Vortex beads before use.

Before first use of the kit

- Download the KingFisher™ Flex script **MVP_Ultra_Flex** from the MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit (Cat. No. A42356) product page, then install it on the instrument.

  See the instrument user guide for instructions to install the script.

- Prepare fresh 80% Ethanol using 100% absolute Ethanol and Nuclease-free Water, sufficient for 1.5 mL per sample, plus 10% overage.

Set up the KingFisher™ Flex instrument

- Ensure that the KingFisher™ Flex instrument has the appropriate magnetic head and heat block installed.
  - 96 deep-well magnetic head
  - 96 deep-well heat block
- Ensure that the **MVP_Ultra_Flex** script is installed on the instrument.

Reconstitute TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*)

Use of the TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*) is optional.

1. Remove metal fastener from vial using tweezers and place vial on ice.
2. Remove rubber stopper from vial, then add 200 µL 1X PBS (1X), pH 7.4 to the vial.
3. Replace the rubber stopper, then vortex the tube to mix.
4. Transfer reconstituted sample to a 1.5-ml tube, then store on ice or at 4°C.

Note: Store the reconstituted control at 4°C for up to 48 hours. For long term storage, store the reconstituted control at –80°C to –20°C for up to 4 months. Mix well to resuspend before use.

The final concentration of the control is 5×10^6 copies/µL.

Set up the processing plates

Set up the processing plates outside the instrument according to the following table. Cover the plates with a temporary seal, then store at room temperature for up to 1 hour while you set up Sample Plate.

Plate type	Plate position	Plate ID	Reagent	Volume per well
Deep well[1]	2	Wash 1 Plate	Wash Solution	1000 µL
	3	Wash 2 Plate	80% Ethanol	1000 µL
	4	Wash 3 Plate	80% Ethanol	500 µL
	5	Elution Plate	Elution Solution	60 µL[2]
Standard[3]	6	Tip Comb	96DW Tip Comb	—

[1] KingFisher™ 96 Deep-Well Plate
[2] The elution volume can be increased to a maximum of 100 µL.
[3] KingFisher™ 96 KF microplate

Set up Sample Plate, then start processing

(*Optional*) Reconstitute TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*) before use in step 3 (see page 17).

1. Swirl the bottle of Enzyme Mix, then place on ice.
2. Add 50 µL of Enzyme Mix to each well in a KingFisher™ 96 Deep-Well Plate (Sample Plate).

3. Add samples and controls to the appropriate well containing Enzyme Mix.

Sample or control	Instructions
Sample	Add 200–400 µL of sample to a well.
Negative Extraction Control (NEC)	Add 200–400 µL of Universal Transport Media to a well.
(*Optional*) TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*)	• Combine 10 µL of reconstituted control with 390 µL of Universal Transport Media in a well. *or* • Add 10 µL of reconstituted control to one or more sample wells.

4. On the KingFisher™ Flex instrument, select the **MVP_Ultra_Flex** script, then press **Start**.

5. Follow the instrument prompts to load sample and processing plates, then press **Start**.

Proceed immediately to the next step.

Continue processing to bind, wash, and elute the nucleic acid

1. During the enzyme treatment incubation on the instrument, prepare the Binding/Bead Mix.
   a. Vortex the tube of Nucleic Acid Binding Beads to fully resuspend the beads.
   b. Combine the following components for the required number of samples, plus 10% overage.

   IMPORTANT! Binding Solution is viscous. Pipet slowly to avoid bubbles and to ensure that the correct volume is delivered.

Component	Volume per sample
Binding Solution	530 µL
Nucleic Acid Binding Beads	20 µL
(*Optional*) TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control	10 µL
Total	**550 µL** or **560 µL**

2. Gently invert the Binding/Bead Mix 5 times to mix, then store at room temperature until the next step.

3. When prompted by the instrument (approximately 20 minutes after the start of the script), remove the Sample Plate from the instrument.

4. Add 10 µL of Proteinase K to each sample in the Sample Plate.

   Note: Add the Proteinase K to the sample separately from and before the Binding/Bead Mix. Combining the reagents, or adding in a different order can affect nucleic acid recovery.

5. Gently invert the Binding/Bead Mix 5 times to mix, then use a manual pipet (single or multi-channel) to dispense the appropriate volume to each sample and control well in the Sample Plate.

   - 550 µL: Binding/Bead Mix only *or*
   - 560 µL: Binding/Bead Mix + TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control

   IMPORTANT! Binding/Bead Mix is viscous. Pipet slowly to avoid bubbles and to ensure that the correct volume is delivered. Invert the Binding/Bead Mix regularly to avoid bead settling.

6. Return Sample Plate to the instrument, then press **Start** to resume the script.

7. When processing is complete (~30 minutes after adding Binding/Bead Mix), remove Elution Plate from instrument.
   The purified nucleic acid is in Elution Plate.

8. Transfer the nucleic acid samples to a 96-well storage plate or seal Elution Plate.

Store nucleic acid samples on ice for immediate use or at –20°C for longer-term storage.

3 Perform preamplification

For required materials, see Table 5 on page 11 and Table 6 on page 12.

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

Perform preamplification

Note: Preamplification of the TrueMark™ Respiratory Panel 2.0 Amplification Control is not recommended.

1. Prepare PreAmp Reaction Mix: Combine the following components for the number of required reactions plus 10% overage, then mix thoroughly by pipetting up and down.

Component	Volume per reaction
TaqPath™ 1-Step RT-qPCR Master Mix, CG	2.5 µL
TrueMark™ Respiratory Panel 2.0 PreAmp Primers[1]	2.5 µL

[1] The TrueMark™ Respiratory Panel 2.0 PreAmp Primers contains primers for all respiratory tract microbiota assays plus the TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control and the RNase P RPPH1 gene, but does not contain primers for the TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*).

2. Distribute PreAmp Reaction Mix, then Nuclease-free Water or sample nucleic acid to the appropriate wells of a 96-well plate.

Component	Sample reaction	No-template control (NTC) reaction
PreAmp Reaction Mix	5 µL	5 µL
Sample DNA or NEC	5 µL	—
Nuclease-free Water	—	5 µL
Total volume per reaction	**10 µL**	**10 µL**

3. Seal the plate with adhesive film.

4. Gently vortex the plate for 10 seconds to mix, then briefly centrifuge to bring contents to the bottom of the wells.

5. Place the plate in a thermal cycler that is programmed with the following thermal cycling conditions, then start the run.

Stage	Step	Temperature	Time
Hold	UNG incubation[1]	25°C	2 minutes
Hold	Reverse transcription	50°C	30 minutes
Hold	Activation	95°C	2 minutes
Cycling (14 cycles)	Denaturation	95°C	15 seconds
	Annealing/Extension	60°C	2 minutes
Hold	Inactivation	99.9°C	10 minutes
Hold	—	4°C	Hold

[1] Heat-labile UNG is completely inactivated during the initial ramp to 95°C.

6. Store the plate on ice until dilution for PCR (see “Dilute the preamplified product” on page 21).

Dilute the preamplified product

To determine dilution volumes, first determine the total volume of diluted preamplified sample that is required for PCR (see Chapter 4, “Prepare and run TaqMan™ Array Cards”). We recommend that you prepare only the volume of diluted preamplified sample that is required for your experiment. The undiluted preamplified sample can be stored at –20°C long term.

1. Vortex, then briefly centrifuge the plate that contains the completed preamplification sample reactions.

2. Remove the adhesive film from the plate.

3. Prepare a 1:20 dilution of the preamplified samples in a new 96-well plate.
    a. Transfer the desired volume of the preamplified samples to a new 96-well plate (for example, 2 µL).
    b. Add the appropriate volume of Nuclease-free Water to each sample and control well (for example, 38 µL).
4. Seal the plate with new adhesive film.
5. Vortex the plate for 10 seconds, then briefly centrifuge.
6. Proceed directly to PCR (see Chapter 4, “Prepare and run TaqMan™ Array Cards”).

Seal the plate that contains the unused portion of the undiluted preamplified product, then store at –20°C.

4 Prepare and run TaqMan™ Array Cards

For detailed instructions for handling TaqMan™ Array Cards, see Appendix C, “Detailed procedures for preparation of a TaqMan™ Array Card”.

Download the plate file for the array card at **thermofisher.com/taqmanfiles**.

1. Allow the card to equilibrate to room temperature.
2. Gently mix the bottle of TaqMan™ Fast Advanced Master Mix, No UNG.

   IMPORTANT! Ensure that you use TaqMan™ Fast Advanced Master Mix, No UNG. If you use a master mix that contains UNG, the preamplified samples will be degraded.
3. For each port, combine the following components.

   (*Optional*) Use 10 µL of TrueMark™ Respiratory Panel 2.0 Amplification Control plus 10 µL of Nuclease-free Water instead of diluted preamplified product, as a positive amplification control sample.

Component	Volume per port
Diluted preamplified product	20 µL
TaqMan™ Fast Advanced Master Mix, No UNG	50 µL
Nuclease-free Water	30 µL
Total volume per port	**100 µL**

4. Fill each port with 100 µL of prepared mix.
5. Centrifuge the card at 1,200 rpm (301 × *g*) for 1 minute.
6. Repeat step 5.
7. Seal the card using TaqMan™ Array Card Sealer (see “Seal the card” on page 41 for detailed instructions).
8. Load the card into the real-time PCR instrument, then set up the experiment in the instrument software.
   - Experiment type—**Array Card**
   - Experiment—**Standard curve**
   - Run type—**Fast**
   - Sample and assay assignments—Import the plate file (TXT) for the card, then assign samples.

- Run method—Change the default run method to the following settings:

Step	Stage	Cycles	Temp.	Time
Activation	1	1	95°C	10 minutes
Amplification	2	40	95°C	3 seconds
			60°C	30 seconds

9. Run the program.

5 Analyze data

Select analysis software

Software	Analysis option	
	C_{rt} analysis	QC metrics
Relative Quantification Application (recommended)[1] Access the application at thermofisher.com/connect.	✓	✓
QuantStudio™ 12K Flex Software	✓	✓
QuantStudio™ Real-Time PCR Software	✓	✓ [2]

[1] To perform data analysis using the application, you must export your data. For detailed instructions about exporting data, see the instrument documentation.

[2] QC metrics are not available for v1.3 or earlier.

Review results

1. In the analysis settings of the software, select the relative C_{rt} method.

   The relative C_{rt} method is recommended for dried-down assays. Dried-down assays can reconstitute at different rates, causing a dip in the early cycles of the baseline. C_{rt} can correct for a variable baseline.

   - In RQ Application:
     a. Click **(Analysis Settings)**.
     b. In the **C_q Settings** tab, select **Use C_{rt}**.
     c. In the **Endogenous controls** tab, select **Skip target normalization**.
     d. Click **Finish**.
   - In QuantStudio™ 12K Flex Software or QuantStudio™ Real-Time PCR Software—select **Analysis Settings ▸ Ct Settings ▸ Algorithm Settings ▸ Relative Threshold**.

2. Review amplification curves (in log or linear view), C_{rt} values, and amplification curve QC metrics (Amp Score and C_q Confidence) for each reaction.

QC metric	Description
Amp Score	A value to indicate the quality of the amplification curve.
C_q Confidence	A value to reflect the reliability of the derived C_q.

3. (*Optional*) Filter data in the order indicated in the following table.

Note: We encourage testing and establishing your own C_{rt} cut-off value and amplification curve QC metrics for each assay to achieve high sensitivity and specificity.

Parameter to examine	Consider filtering out sample data using the following cut-off values
C_{rt}	$C_{rt} > 30$
Amp Score	Amp Score < 1.2 **Note:** The RNase P (RPPH1) assay has a cut-off value of < 1.0
C_q Confidence	C_q Confidence < 0.7 **Note:** The following assays have a cut-off value of < 0.5 • RNase P (RPPH1) • Human Rhinovirus 1/2 (RV_1of2) • Human Rhinovirus 2/2 (RV_2of2) • Bordetella holmesii (B. holmesii)

Note: The C_{rt} method is recommended for analysis. If you select the comparative C_t method, use the following analysis settings. Review real-time data as described above.

- Baseline—Auto
- Threshold—0.2 C_t

Considerations for data analysis

Organisms that are covered by more than one assay

- For full strain coverage of adenoviruses or rhinoviruses, two assays are used. A positive result with either or both of the assays indicates a positive result for the organism.
- For SARS-CoV-2 detection, assays to the S-protein and N-protein genes each have high coverage of known strains. A positive with either or both assays indicates a positive result for SARS-CoV-2.

Species-specific assays that are also covered by pan or broad coverage assays

Species-specific assay	Considerations for data analysis
Flu A assays	• The Flu_A_pan assay detects Influenza A H1 and H3, for which there are also strain-specific assays. • Samples that are positive for the Flu_A_H1 or Flu_A_H3 assay typically are positive for the Flu_A_pan assay.

(continued)

Species-specific assay	Considerations for data analysis
Bordetella assays	• The Bordetella assay detects *B. pertussis*, *B. bronchiseptica*, and *B. parapertussis* strains. Strain-specific assays for *B. pertussis* and *B. holmesii* are also available. • Most samples that are positive for the *B. pertussis* assay are also positive with the Bordetella assay.
RSV assays	• Samples that are positive for the RSVA assay may be detected at a lower efficiency (a difference of several C_{rt} values) by the RSVB assay.
Enterovirus (EV) and rhinovirus (RV) assays	• The RV assays detect both RV and EV strains whereas the EV assays are specific for EV strains. Thus enterovirus positive samples are detected by both EV and RV assays whereas rhinovirus positive samples are detected only by the RV assays. • The EV_pan assay detects all human enterovirus species except D68, for which there is a strain-specific EV_D68 assay. • Samples that are positive for the EV_D68 assay may be detected at a lower efficiency (a difference of several C_{rt} values) by the EV_pan assay.
Bacterial and HHV assays	• It is not unusual to detect *M. catarrhalis*, *H. influenzae*, *K. pneumoniae*, *S. pneumoniae*, and *S. aureus* in respiratory samples as these are commensal or transiently commensal upper respiratory tract microbes. • Due to the high prevalence of human infection with HHV4 (EBV) and HHV6 viruses, these viruses can be detected at low levels in some respiratory samples.

Approximate C_{rt} range for controls

If used as recommended in this guide, the approximate C_{rt} range for control assays are as follows.

Control	Approximate C_{rt} range
TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control	20-23
TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*)	23-27
TrueMark™ Respiratory Panel 2.0 Amplification Control	20-23

A

Troubleshooting

Troubleshooting: Nucleic Acid Isolation

Observation	Possible cause	Recommended action
Beads remain in sample after elution	Excessive amount of recovered host genomic DNA/RNA is preventing nucleic acid separation from the beads.	Increase elution volume to 100 µL.
		Reduce the input volume of starting sample to 200 µL.
Reduced extraction efficiency of TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control	Proteinase K enzyme was either omitted from the sample or added incorrectly.	Always add Proteinase K enzyme to the sample separately and before adding the Binding/Bead Master mix.
	TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control added at the wrong step.	Ensure that the TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control is added to the Binding/Bead Master mix before dispensing into sample wells.

Troubleshooting: After removing the card from packaging

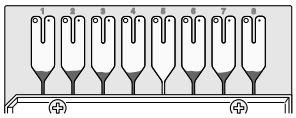
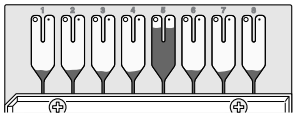
Observation	Possible cause	Recommended action
Water condenses on the reaction wells (optical side of the card)	The card was not at room temperature before being removed from the packaging.	Remove condensation on the reaction wells by lightly blowing room temperature pressurized nitrogen or an air blower on the wells. **IMPORTANT!** Ensure that all water condensation is removed. The optical side of the card must be free of water condensation.

Troubleshooting: After loading PCR reaction mix into the card

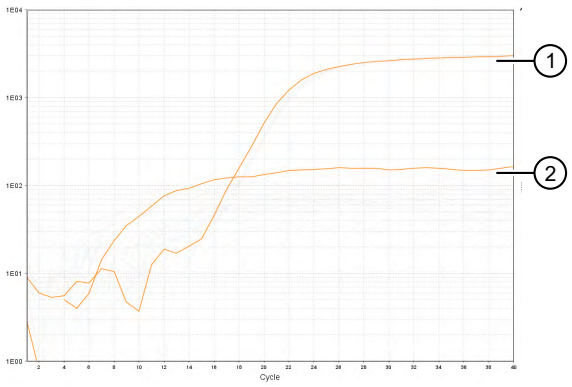
Observation	Possible cause	Recommended action
Fill reservoirs have bubbles in the PCR reaction mix	When loading the card with PCR reaction mix, air was introduced into the fill reservoir.	Inspect the affected rows after centrifuging and sealing the card. Note wells that contain bubbles, then consider omitting these wells from analysis.

Observation	Possible cause	Recommended action
Fill reservoir is not full of PCR reaction mix	The PCR reaction mixture was not correctly pipetted into the fill reservoir.	Be sure to correctly pipette the entire PCR reaction mixture (100 µL) into the fill reservoir. Add more sample-specific PCR reaction mix to the fill reservoir.
PCR reaction mix leaks from the vent port into the fill reservoir	The PCR reaction mixture was not correctly pipetted into the fill reservoir.	Be sure to correctly pipette the entire PCR reaction mixture (100 µL) into the fill reservoir. Add more sample-specific PCR reaction mix to the fill reservoir.

Troubleshooting: After centrifuging the card

Observation	Possible cause	Recommended action
Fill reservoir completely empty 	Some wells were filled improperly.	Continue with running the card. However, consider omitting the wells associated with that fill reservoir.
PCR reaction mix remains in a fill reservoir 	Though rare, the fill port is blocked.	Inspect the card for blocked fill port or a pinched channel. If the fill reservoir is defective, contact Support.
	Filling is incomplete or not consistent.	Centrifuge the card again for 1 minute.
		If the filling is still incomplete after the additional centrifuge cycle, continue with running the card. However, consider omitting the wells associated with that fill reservoir.

Troubleshooting: After running the card and reviewing run results

Observation	Possible cause	Recommended action
Unexpected C_{rt} values in the amplification plot  ① Expected C_{rt} value (noted in most replicates) ② Unexpected C_{rt} value (too low)	Unexpectedly low C_{rt} values (<10) — Signal variation or noise in early PCR cycles	Review amplification curves, Amp Score, and C_q Confidence values.
		Consider filtering C_{rt} values from analysis.
		Compare replicates, if available.
		Dilute the samples, then repeat the experiment.
	Unexpectedly high C_{rt} values (27–30) — Sporadic amplification	Review amplification curves, Amp Score, and C_q Confidence values.
		Compare replicates, if available.
		Repeat the experiment.
No amplification in some wells	Empty wells due to improper card sealing.	When sealing the card, use a slow and steady motion to push the carriage across the TaqMan™ Array Card Sealer. **IMPORTANT!** Do not move the carriage back across the card. See "Seal the card" on page 41.
	Positive sample is not present in the wells.	No action required. Consider using one or more positive controls to confirm nucleic acid recovery.
No amplification for portions of the card	The card was misaligned in the block during the instrument run.	Inspect the card for crushed or distorted feet. If there are damaged feet, contact Support.  ① Array card feet
	Positive sample is not present in the wells.	No action required. Consider using one or more positive controls to confirm nucleic acid recovery.

Observation	Possible cause	Recommended action
No amplification within or across one or more rows	Empty wells due to improper card sealing.	When sealing the card, use a slow and steady motion to push the carriage across the TaqMan™ Array Card Sealer.
	Empty wells due to misalignment of the TaqMan™ Array Card Sealer.	If the TaqMan™ Array Card Sealer is misaligned, contact Support.
	PCR reaction mix improperly prepared.	Ensure that all reaction components were added to the PCR reaction mix.
	Positive sample is not present in the wells.	No action required. Consider using one or more positive controls to confirm nucleic acid recovery.
The baseline is variable	The dried-down assays on the card were reconstituted at different rates, causing a dip in the early cycles of the baseline.	Use the relative threshold algorithm (C_{rt}). C_{rt} can correct for a variable baseline.
		Use the Relative Quantification application, available on the Connect. The Relative Quantification application uses C_{rt} if the software specific to your instrument does not have the relative threshold algorithm.
Noise in the amplification plots for portions of the card	The card was misaligned in the block during the instrument run.	Inspect the card for crushed or distorted feet. If there are damaged feet, contact Support.  ① Array card feet
A gradient signal across the card	The card was in a diagonal position during centrifugation because not all of the positions in the card holder were filled.	Repeat the assay with a new card. Ensure that all the positions in the centrifuge card holder are filled.

B

1-step RT-PCR procedure

For maximum sensitivity, we strongly recommend using the 2-step procedure as described in Chapter 4, “Prepare and run TaqMan™ Array Cards”.

The following 1-step procedure can be used if sensitivity requirements can be met without using preamplification.

Note: This 1-step procedure has not been fully optimized for respiratory tract microorganism detection.

Prepare and run TaqMan™ Array Cards (alternate 1-step RT-PCR procedure)

The same thermal cycling program can be used for both RNA and DNA targets. The reverse transcription step does not affect performance with DNA targets.

For detailed instructions for handling TaqMan™ Array Cards, see Appendix C, “Detailed procedures for preparation of a TaqMan™ Array Card”.

1. Allow the card to equilibrate to room temperature.
2. Gently mix the bottle of TaqPath™ 1-Step RT-qPCR Master Mix, CG.
3. For each port, combine the following components.

Component	Volume per port
Nucleic acid[1]	50 µL
TaqPath™ 1-Step RT-qPCR Master Mix, CG	25 µL
Nuclease-free Water	25 µL
Total volume per port	**100 µL**

[1] For more information, see Chapter 2, “Isolate nucleic acid using MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit”.

10–50 µL of nucleic acid sample can be used. The ratio of nucleic acid sample to water can be adjusted based on:

- Sample availability
- Need for sample archiving
- Sample splitting for port replicates

4. Fill each port with 100 µL of prepared mix.
5. Centrifuge the card at 1,200 rpm for 1 minute.

6. Repeat step 5.
7. Seal the card using TaqMan™ Array Card Sealer.
8. Load the card into the real-time PCR instrument, then set up the experiment in the instrument software.

   Settings:

   - Experiment type—**Array Card**
   - Experiment—**Standard Curve**
   - Run type—**Fast**
   - Sample and assay assignments—Import the plate file (TXT) for the card, then assign samples
   - Run method—Change the default run method to the following settings:

Step	Stage	Cycles	Temperature	Time
UNG incubation[1]	1	1	25°C	2 minutes
Reverse transcription	2	1	50°C	15 minutes
RT inactivation/denaturation	3	1	95°C	10 minutes[2]
Amplification	4	40	95°C	3 seconds
			60°C	30 seconds

[1] Heat-labile UNG is completely inactivated during the initial ramp to 95°C.
[2] To completely dissolve the primers and probes on the card.

9. Run the program.

Data analysis parameters for 1-step RT-PCR

We encourage testing and establishing your own C_{rt} cut-off value and amplification curve QC metrics for each assay to achieve high sensitivity and specificity.

Parameter to examine	Consider filtering out sample data using the following cut-off values
C_{rt}	$C_{rt} > 35$
Amp Score	Amp Score < 1.2 **Note:** The RNase P (RPPH1) assay has a cut-off value of < 1.0
C_q Confidence	C_q Confidence < 0.7 **Note:** The following assays have a cut-off value of < 0.5 • RNase P (RPPH1) • Human Rhinovirus 1/2 (RV_1of2) • Human Rhinovirus 2/2 (RV_2of2) • Bordetella holmesii (B. holmesii)

For more information about reviewing the results, see the following sections:

- "Review results" on page 25
- "Considerations for data analysis" on page 26

C Detailed procedures for preparation of a TaqMan™ Array Card

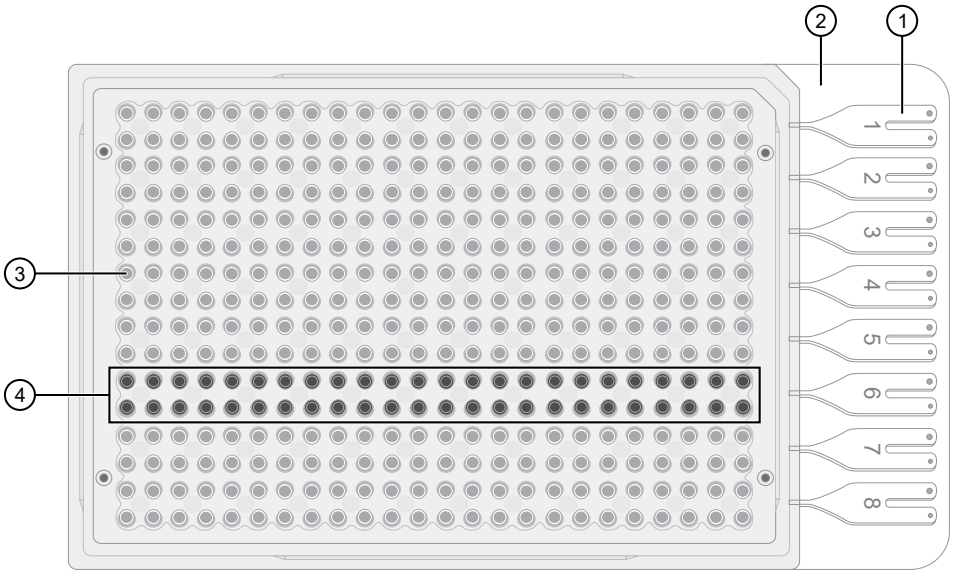
TaqMan™ Array Card overview

TaqMan™ Array Cards are 384-well microfluidic cards that are prepared with dried-down TaqMan™ Assays.

Advantages of using TaqMan™ Array Cards include:

- Small-volume design that minimizes sample and reagent consumption.
- Streamlined reaction setup that saves time and reduces labor-intensive steps.
- Access to high-throughput, 384-well format without liquid-handling robotics.
- Two-fold discrimination detection at the 99.7% confidence level.
- Standardization across multiple samples in multiple laboratories.

Each card can run 1 to 8 samples against 12 to 384 TaqMan™ Assay targets (including controls).



① **Fill reservoir**—Each reservoir is loaded with a sample-specific PCR reaction mix; the associated reaction wells fill with that sample (8 total reservoirs)

② **Fill reservoir strip**—Support strip for fill reservoirs; removed before running the card

③ **Reaction well**—Each well contains dried-down assay (384 total reaction wells)

④ **Reaction well row**—A set of reaction wells that fill with the same sample-specific PCR reaction mix (8 total rows, each row associated with a single fill reservoir)

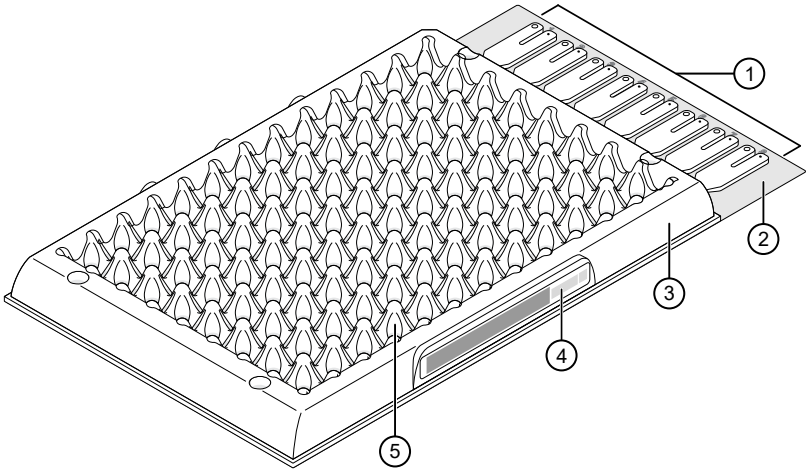
Guidelines for preparation of a card

- Keep the card protected from light and stored as indicated until ready for use. Excessive exposure to light may affect the fluorescent probes of the dried-down assays in the card.
- Before removing the card from its packaging:
  - Prepare each sample-specific PCR reaction mix.
  - Allow the card to reach room temperature.
- Load each fill reservoir with 100 µL of sample-specific PCR reaction mix.
  - Each fill reservoir contains a single sample as determined by the card layout.
  - The 100–µL volume ensures adequate filling of each reaction well. Volumes smaller than 100 µL result in insufficiently filled cards.
- Do not allow the micropipette tip to contact the coated foil beneath the fill port.

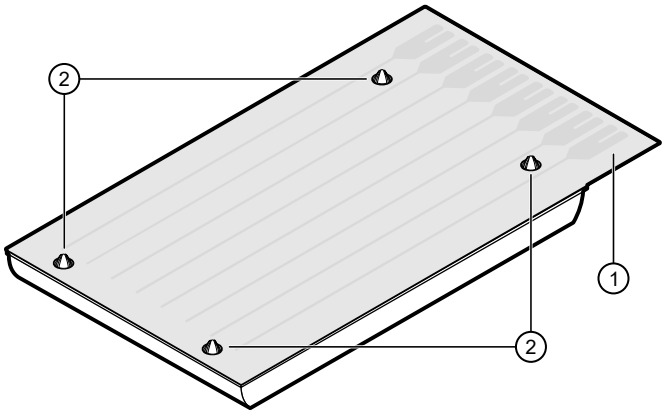
- Load the card with PCR reaction mix *before* centrifuging the card.
  During centrifugation, the PCR reaction mix resuspends the dried-down assays in each well of the card. Adding sample after centrifuging disrupts the assay layout of the card.
- Run the card within the time allowed by the Master Mix.
- If the card is not run immediately, protect it from light and store at 2-8°C.

TaqMan™ Array Card diagram

A TaqMan™ Array Card includes 8 fill reservoirs and 384 reaction wells.

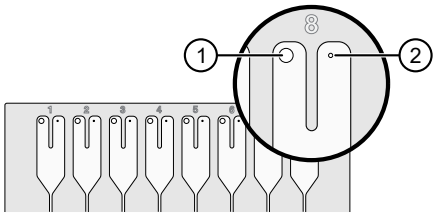


1. Fill reservoirs (8 total)
2. Fill reservoir strip
3. Array card carrier
4. Array card barcode
5. Reaction well (384 total)



1. Foil
2. Array card feet

The fill reservoir includes a fill port and a vent port. Use the fill port to load PCR reaction mix into the card.



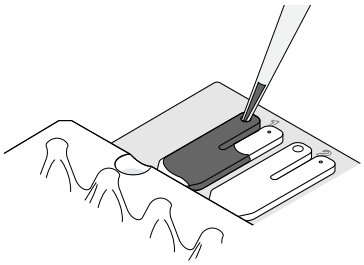
① Fill port
② Vent port

Load the PCR reaction mix

Before removing the card from its packaging:

- Prepare each sample-specific PCR reaction mix.
- Allow the card to reach room temperature.

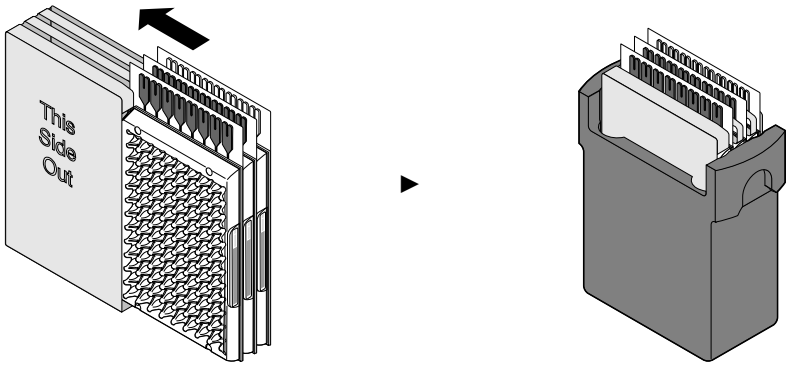
1. Carefully remove the card from its packaging.
2. Place the card on the benchtop with its foil-side down.
3. Load 100 µL of the sample-specific PCR reaction mix into a micropipette.
4. Hold the micropipette in an angled position, then place the tip into a fill port of the card.
5. Slowly dispense the entire volume of reaction mix so that it sweeps in and around the fill reservoir toward the vent port.



Centrifuge the card

1. Load the cards into the centrifuge buckets.
   a. Place the bucket on the benchtop with its label facing the front of the bench.
   b. Insert the cards into the card holder, ensuring that:
      - The fill reservoirs extend upwards out of the card holder.
      - The reaction wells face the label-side of the card holder.
   c. Insert blank balance cards into any empty positions of the card holder. All three positions in the card holder must be filled.

d. Place the loaded card holder into the bucket so that the card holder label faces the front of the bucket.



2. Configure the centrifuge using its front-panel controls.

   a. Set the bucket type to **15679**.

   b. Set the following parameters according to the control panel type.

Parameter	EASYSet (touchpad-operated)	QUIKSet (knob-operated)
Increasing ramp rate	9	3
Decreasing ramp rate	9	—
Rotational speed	1,200 rpm (331 × *g*)	1,200 rpm
Centrifugation time [1]	1 minute	1 minute

[1] You will centrifuge the cards twice, each time for 1 minute (see step 4).

IMPORTANT! A speed that is set too high can deform the card.

3. Load the buckets into the centrifuge.

   a. Press ⏏ on the centrifuge control panel to open the centrifuge cover.

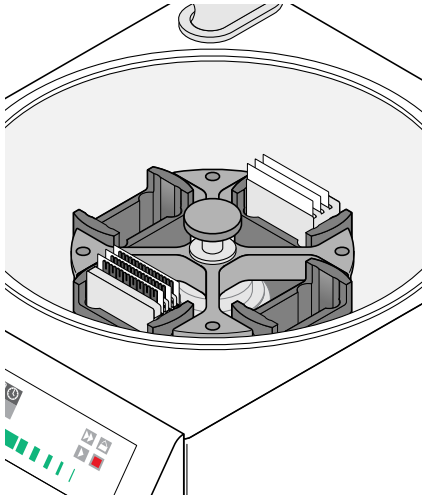
   b. Place each loaded bucket onto an open rotor arm of the centrifuge.

   Ensure that each bucket can swing easily within its slotted position on the rotor arm.

   c. If there are empty rotor arms, prepare buckets with blank balance cards as described in step 1. Place the balance buckets onto the rotor arms.

   The rotor must be evenly loaded and opposing buckets must hold the same weight.

   d. Close the centrifuge cover.



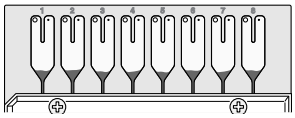
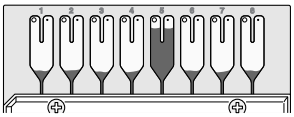
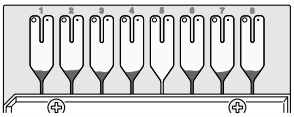
Centrifuge is properly loaded and balanced.

4. Run the centrifuge.
   a. Press ▶.
      The centrifuge will start, then automatically stop after 1 minute.
   b. Repeat substep 4a so that the cards are centrifuged for a total of two, consecutive, 1-minute centrifugations.

      IMPORTANT! Do not centrifuge the cards continuously for 2 minutes. The ramping up in speed during the *two, consecutive 1-minute* centrifugations is important for proper filling.

5. Remove the cards from the centrifuge.
   a. Press ⏏.
   b. Remove the buckets from the centrifuge, then remove the card holders from the buckets.
   c. Remove each card from the card holder by lifting it gently by the card carrier sides.

6. Examine the cards for proper filling.
   When properly filled, the remaining volumes of PCR reaction mix are consistent reservoir to reservoir.
   If the card is not properly filled, see Appendix A, “Troubleshooting”.

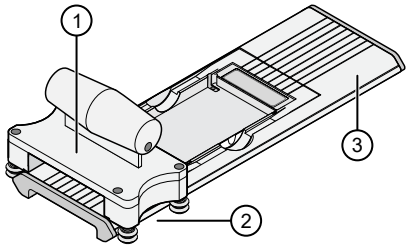
Consistent filling	Inconsistent filling	
		

Seal the card

The TaqMan™ Array Card Sealer isolates the wells of an array card after it is loaded with PCR reaction mix and centrifuged. The sealer uses a precision stylus assembly (under the carriage) to seal the main fluid distribution channels of the array card.

Note: In some documents, the TaqMan™ Array Card Sealer is referred to as a Stylus Staker.

1. Position the TaqMan™ Array Card Sealer and its carriage before inserting a card.
   a. Place the sealer on a benchtop that is approximately waist-high so that the carriage can be easily maneuvered.
   b. Position the sealer with the carriage starting position toward the front of the bench.
      Ensure that the engraved arrows on the sealer point away from you.

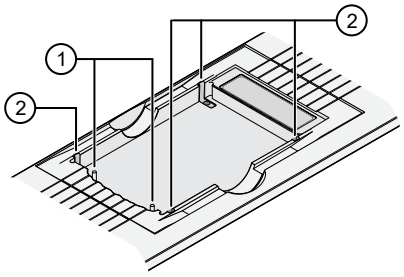


① Carriage
② Carriage starting position
③ Carriage ending position

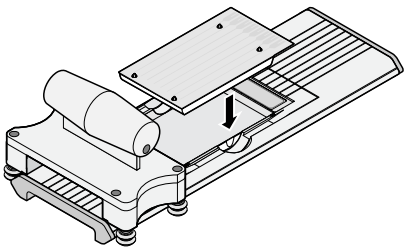
   c. Ensure that the carriage for the sealer is at the starting position.

IMPORTANT! Do not insert a filled card into the sealer if the carriage is *not* in its starting position. The card will be irreparably damaged if the carriage is moved backwards across the card towards the starting position.

2. Insert a filled, centrifuged card into the sealer.
   a. Hold the card with its foil-side up.
   b. Orient the card over the sealer with the fill reservoirs of the card toward the ending position.
   c. Align the rear pin grooves of the card to the alignment pins of the sealer.



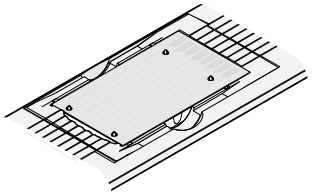
① Alignment pins
② Spring clips



   d. Gently place the card on top of the sealer.

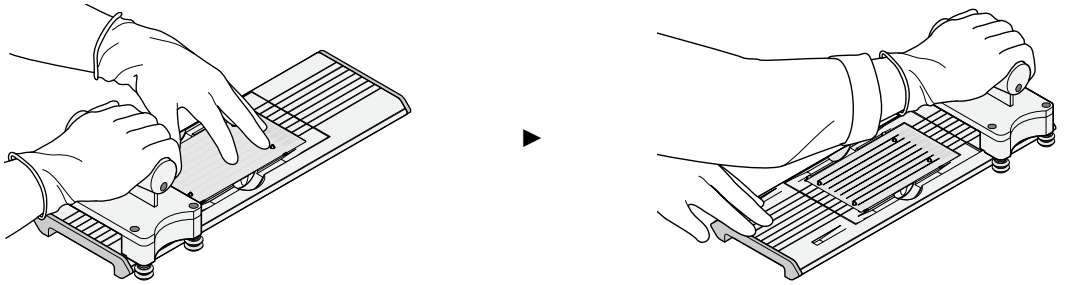
e. Gently push the card until it is seated securely in the sealer.

   When properly seated, the foil surface of the card is level with the base of the sealer and the spring clips hold the card securely in place.



3. Slowly and steadily push the carriage across the sealer in the direction of the engraved arrows.

   Push the carriage across the entire length of the card until the carriage meets the mechanical stops at the ending position.



IMPORTANT!

- Do not use excessive force or speed when pushing the carriage across the card.
- Do not move the carriage back across the card. Leave the carriage at the ending position while removing the card from the sealer.

4. Remove the sealed card from the sealer by grasping the sides of the card and lifting it off.

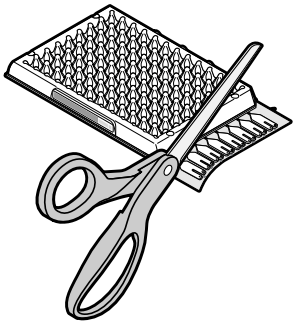
   Use the thumb slot in the middle of the sealer to access the card.

5. Examine the card for proper sealing.

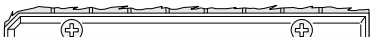
Note: When properly sealed, the indentations from the sealer carriage align with the main channels of the card.

If the indentations do not align or if the foil is damaged, do not use the card.

6. Use scissors to trim the fill reservoir strip from the card. Use the edge of the card carrier as a guide.



IMPORTANT! Completely remove the fill reservoir strip. Any remaining plastic that extends beyond the card edge can prevent the card from seating properly on the sample block and can affect amplification.

Correct trim	Incorrect trim
	

The card is now ready to run on the instrument.

Run the card within the time allowed by the Master Mix.

D

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, see the "Documentation and Support" section in this document.

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!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

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)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Publication Number
Isolation of Nucleic Acid for Respiratory Tract Microbiota Profiling Experiments Quick Reference	MAN0018526
Respiratory Tract Microbiota Profiling Experiments v2 using TaqMan™ Array Cards Quick Reference	MAN0019508
TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card Product Information Sheet	MAN0019511
TrueMark™ Respiratory Panel 2.0 Amplification Control Product Information Sheet	MAN0019505
TaqMan™ Universal Extraction Control Organism (B. atrophaeus) Product Information Sheet	MAN0018535
TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control Product Information Sheet	MAN0018534
QuantStudio™ 12K Flex Real–Time PCR System: Multi-Well Plates and Array Card Experiments User Guide	4470935
QuantStudio™ 12K Flex Real–Time PCR System Maintenance and Administration Guide	4470689
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide	4489821
Thermo Scientific™ KingFisher™ Flex User Manual	N07669
TaqPath™ 1-Step RT-qPCR Master Mix, CG User Guide	MAN0007959

Customer and technical support

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- 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**.