

Respiratory Tract Microbiota Profiling Experiments

APPLICATION GUIDE

TaqMan™ Assays for respiratory tract microbiota profiling
experiments using OpenArray™ plates

for use with:

TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate

Custom TaqMan™ OpenArray™ Plate

MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit

QuantStudio™ 12K Flex instrument with OpenArray™ block (AccuFill™ System)

Catalog Number A41237

Publication Number MAN0017952

Revision C.0



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

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0017952

Revision	Date	Description
C.0	4 January 2021	<ul style="list-style-type: none"> Updated instructions to order Custom TaqMan™ OpenArray™ Plates. Corrected information for TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate (see Table 3 on page 9). Provided additional information about the number of samples and controls. Updated the descriptions of the controls. Updated the preamplification reagent to Megaplex™ Preamp Primers, RTM (Cat. No. A41374). Specified that the KingFisher™ Flex script is downloaded from the MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit product page. Added instructions to review the amplification plot when reviewing results. Added approximate C_t ranges for controls. Corrected the name of the plate in “OpenArray™ plate assembly and handling errors” on page 43.
B.0	09 September 2019	<ul style="list-style-type: none"> Updated product information for TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate and controls Updated data analysis recommendations Updated nucleic acid isolation kit product information and workflow Removed Early Access designation
A.0	09 November 2018	New document.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Trademarks: All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a registered trademark of Roche Molecular Systems, Inc., used under permission and license. Biomek is a trademark of Beckman Instruments, Inc. Beckman Coulter is a trademark of Beckman Coulter, Inc.

©2021 Thermo Fisher Scientific Inc. All rights reserved.

Contents

■ CHAPTER 1 Overview	6
Product description	6
TaqMan™ assays for respiratory tract microbiota profiling	6
TaqMan™ assays included in the TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate	6
Additional TaqMan™ assays for respiratory tract microbiota profiling	8
TaqMan™ OpenArray™ Plate products and formats	9
TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate	9
Custom TaqMan™ OpenArray™ Plate formats	9
Materials required but not supplied	11
Materials required for nucleic acid isolation	11
Materials required for preamplification	12
Materials required for OpenArray™ plate workflow	13
Materials required for data analysis	14
Optional controls	14
TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control	14
TaqMan™ Universal Extraction Control Organism (<i>B. atrophaeus</i>)	15
TaqMan™ Respiratory Tract Microbiota Amplification Control	15
Workflow	16
■ CHAPTER 2 Isolate nucleic acid using MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit	17
Procedural guidelines	17
Before first use of the kit	17
Set up the KingFisher™ Flex instrument	17
Reconstitute TaqMan™ Universal Extraction Control Organism (<i>B. atrophaeus</i>)	18
Set up the processing plates	18
Set up Sample Plate, then start processing	18
Continue processing to bind, wash, and elute the nucleic acid	19

■	CHAPTER 3	Perform preamplification	21
		Good laboratory practices for PCR and RT-PCR	21
		Perform preamplification	21
		Dilute the preamplified sample	22
■	CHAPTER 4	Prepare and run respiratory tract microbiota profiling experiments with OpenArray™ plates	24
		Workflow: Microbiota profiling experiments with OpenArray™ plates	25
		One-time procedures	25
		Set up default folders and software preferences	25
		Obtain TPF files	27
		Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software	27
		Set up the PCR reactions in an OpenArray™ 384-well Sample Plate	28
		Set up the AccuFill™ instrument and software	29
		Transfer reactions to the OpenArray™ plate using the AccuFill™ instrument	30
		Seal the OpenArray™ plate	31
		Run the OpenArray™ plate on the QuantStudio™ 12K Flex Instrument	32
		Check the quality-control images	33
■	CHAPTER 5	Analyze data	35
		Select analysis software	35
		Export data (if necessary)	35
		Review results	36
		Considerations for data analysis	37
		Approximate C _{rt} range for controls	38
		Fields for reviewing results with pivot tables	38
■	APPENDIX A	Troubleshooting	40
		Troubleshooting: Nucleic Acid Isolation	40
		Troubleshoot unexpected C _{rt} values	40
		Troubleshoot with cycling and imaging run images (QC images)	41
		AccuFill™ instrument plate loading errors	42
		OpenArray™ plate assembly and handling errors	43
■	APPENDIX B	Safety	45
		Chemical safety	46
		Biological hazard safety	47

■ APPENDIX C	Documentation and support	48
	Related documentation	48
	Symbols that may be displayed on the instrument, in the software, or in this guide	48
	Customer and technical support	49
	Limited product warranty	49



Product description

TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate (Cat. No. A41237) is an efficient, easy-to-use fixed-content OpenArray™ plate for the characterization of key respiratory tract microbial targets. The plate includes TaqMan™ assays that have been optimized for detection of respiratory tract viral and bacterial nucleic acid. The plate also includes control assays for TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*), TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control, and the human RNase P RPPH1 gene. For a complete list of assays included in the plate, see “TaqMan™ assays included in the TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate” on page 6.

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 have undergone 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 TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate

The following TaqMan™ assays are included in the TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate.

The assays can also be ordered in a Custom TaqMan™ OpenArray™ Plate.

Table 1 Assays for respiratory tract microbiota

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

Table 1 Assays for respiratory tract microbiota (continued)

Target organism	Assay name	Nucleic acid type	Assay ID
<i>Klebsiella pneumoniae</i>	K. pneumoniae	DNA	Ba04932083_s1
<i>Legionella pneumophila</i>	L.pneumophila	DNA	Ba06439617_s1
<i>Mycoplasma pneumoniae</i>	M.pneumoniae	DNA	Ba06439620_s1
<i>Staphylococcus aureus</i>	S.aureus	DNA	Ba04646259_s1
<i>Streptococcus pneumoniae</i>	S.pneumoniae	DNA	Ba06439619_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 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
Human herpesvirus 3 (HHV3 – Varicella zoster Virus)	HHV3	DNA	Vi06439647_s1
Human herpesvirus 4 (HHV4 – Epstein-Barr Virus)	HHV4	DNA	Vi06439675_s1

Table 1 Assays for respiratory tract microbiota (continued)

Target organism	Assay name	Nucleic acid type	Assay ID
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

Table 2 Assays for respiratory tract microbiota controls

Control name	Assay name	Nucleic acid type	Assay ID
TaqMan™ Universal Extraction Control Organism (<i>B. atrophaeus</i>)	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

Additional TaqMan™ assays for respiratory tract microbiota profiling

The following TaqMan™ assays are not included in the TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate. For respiratory tract microbiota profiling using these assays, use a Custom TaqMan™ OpenArray™ Plate.

Target organism	Assay name	Nucleic acid type	Assay ID
Bacteria			
<i>Bordetella holmesii</i>	B.holmesii	DNA	Ba06439621_s1
<i>Coxiella burnetii</i>	C.burnetii	DNA	Ba06439618_s1
<i>Moraxella catarrhalis</i>	M.catarrhalis	DNA	Ba06439622_s1
Fungus			
<i>Pneumocystis jirovecii</i>	P.jirovecii	DNA	Fn06439626_s1
Virus			
Human Parechovirus	HPeV	RNA	Vi99990006_po
Measles virus	Measles	RNA	Vi99990013_po
Middle East Respiratory Syndrome coronavirus (MERS)	MERS_CoV	RNA	Vi06439644_s1

(continued)

Target organism	Assay name	Nucleic acid type	Assay ID
Mumps virus	Mumps	RNA	Vi06439657_s1
Severe Acute Respiratory Syndrome coronavirus (SARS)	SARS_CoV	RNA	Vi06439634_s1

TaqMan™ OpenArray™ Plate products and formats

TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate

The TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate (Cat. No. A41237) contains pre-plated, dried down TaqMan™ assays for respiratory tract microbiota profiling. For the complete lists of assays included with the plate, see “TaqMan™ assays included in the TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate” on page 6.

Contents and storage

Table 3 TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate (Cat. No. A41237)

Component	Amount	Array format	Storage
TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate	1 plate	112	-25°C to -15°C

Assays to the 33 microbial targets and the TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control are plated in triplicate. Assays to the TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*) and human RNase P RPPH1 gene control are plated in duplicate. Each TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate can be used to run 23 samples and one control sample.

Custom TaqMan™ OpenArray™ Plate formats

Custom TaqMan™ OpenArray™ Plate contain pre-plated, dried down TaqMan™ assays for respiratory tract microbiota profiling.

Array format	Number of assays	Maximum number of samples
56	56	48
112	112	24
168	168	16

Note: We recommend at least three technical replicates of each reaction.

Configure and order Custom TaqMan™ OpenArray™ Plates

1. Go to thermofisher.com/order/custom-array.
2. For array type, select **TaqMan™ OpenArray™ Real-Time PCR Inventoried Assays Format**.
3. (Optional) In the table, click **View Layout** to preview the layout of the plate.
4. In the table, click **Select** to configure a plate with the desired array format. The **Custom Array Configurator** screen displays.

Custom Array Configurator

Q Search For Assays **Import Your Assay List** (2) (4) Complete Your Design >

Array name*	Array ID	Array type	Format	Unique Targets	Filled	Invalid	Empty
<input type="text" value="Name your array"/>	-	TaqMan® OpenArray® Real-Time PCR Inventoried Assays Format	18	0	0	0	18

Select Edit Move Export Help Save Your Array (3) Save A Copy...

Click to select assays | Click & drag to move assays | Ctrl+C to copy an assay | Ctrl+V to paste an assay

Display Assay Target

a1							
a2							
a3							
a4							
a5							

Sub Array A1

Filled 0

Invalid 0

Empty 18

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 plate.

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](https://www.thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](https://www.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™ Deep-Well 96 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 (<i>B. atrophaeus</i>)	10010023

Materials required for preamplification

Item	Source
Instrument and equipment	
Thermal cycler, one of the following (or equivalent): <ul style="list-style-type: none"> Veriti™ Thermal Cycler, 96-well standard block SimpliAmp™ Thermal Cycler ProFlex™ PCR System 	Contact your local sales office
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

(continued)

Item	Source
Disposable gloves	MLS
Reagents	
Genomic DNA/RNA	See page 17
Megaplex™ Preamp Primers, RTM	A41374
TaqPath™ 1-Step RT-qPCR Master Mix, CG	A15299
Nuclease-free Water	AM9937 , or equivalent

Materials required for OpenArray™ plate workflow

Item	Source
Instruments, software, and equipment	
OpenArray™ Sample Tracker Software	— ^[1]
QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0	A24945
QuantStudio™ 12K Flex Real-Time PCR Instrument with OpenArray™ block (AccuFill™ System)	4471090
Centrifuge, capable of spinning sample plates at 1,000 rpm	MLS
Plates and accessories	
OpenArray™ 384-well Sample Plates, black	4482221
Biomek™ Seal and Sample Foil Lids (for pre-plating step)	Beckman Coulter™ 538619
OpenArray™ AccuFill™ System Tips	4458107
QuantStudio™ 12K Flex OpenArray™ Accessories Kit ^[2]	4469576
Forceps	MLS
Reagents	
Preamplified genomic DNA/RNA	See page 21
(Optional) TaqMan™ Respiratory Tract Microbiota Amplification Control	A39178
OpenArray™ plates with TaqMan™ Assays	<ul style="list-style-type: none"> • A41237 • Custom ordered^[3]

(continued)


Item	Source
TaqMan™ OpenArray™ Real-Time PCR Master Mix	4462164
Ethanol	MLS

[1] Included with the QuantStudio™ 12K Flex Software.

[2] Each kit contains the items needed to assemble up to 10 plates: 12 lids and plugs, 12 immersion fluid syringes, and 2 carriers. Each custom OpenArray™ plate order is shipped with accessories kits.

[3] See “CustomTaqMan™ OpenArray™ Plate formats” on page 9.

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

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 (<i>B. atrophaeus</i>)	Lyophilized organism control for nucleic acid extraction and purification	Nucleic acid isolation: Stand-alone sample, or add to samples after Enzyme Mix	A39180
TaqMan™ Respiratory Tract Microbiota Amplification Control	DNA plasmid control for real-time PCR	Real-time PCR: Stand-alone sample added directly to the plate	A39178

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 TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate.

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.

TaqMan™ Respiratory Tract Microbiota Amplification Control

TaqMan™ Respiratory Tract Microbiota Amplification Control contains a linearized multi-target plasmid with target sequences for each available respiratory tract microbiota profiling assay. The plasmid also contains target sequences for the TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control, the 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.

TaqMan™ Respiratory Tract Microbiota Amplification Control is supplied at a concentration of 1×10^5 copies/ μL . During real-time PCR, 2.5 μL of the control is used as a stand-alone sample in 2 wells of the TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate. 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

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



Isolate nucleic acid using MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit (page 17)



Perform preamplification (page 21)



Prepare and run respiratory tract microbiota profiling experiments with OpenArray™ plates
(page 24)



Analyze data (page 35)



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

For required materials, see “Materials required for nucleic acid isolation” on page 11.

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™ Deep-Well 96 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 18).

1. Swirl the bottle of Enzyme Mix, then place on ice.
2. Add 50 µL of Enzyme Mix to each well in a KingFisher™ Deep-Well 96 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 (<i>B. atrophaeus</i>)	<ul style="list-style-type: none"> 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.
- Vortex the tube of Nucleic Acid Binding Beads to fully resuspend the beads.
 - 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 “Materials required for preamplification” 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 TaqMan™ Respiratory Tract Microbiota 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
Megaplex™ Preamp Primers, RTM ^[1]	2.5 µL

^[1] Megaplex™ Preamp Primers, RTM contains primers for all of the respiratory tract microbiota assays, plus the TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control and the RNase P RPPH1 gene. It does not contain primers for the TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*).

- 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

- Seal the plate with adhesive film.
- Gently vortex the plate for 10 seconds to mix, then briefly centrifuge to bring contents to the bottom of the wells.
- 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.

- Store the plate on ice until dilution for PCR (see “Dilute the preamplified sample” on page 22).

Dilute the preamplified sample

To determine dilution volumes, first determine the total volume of diluted preamplified sample that is required for PCR (see Chapter 4, “Prepare and run respiratory tract microbiota profiling experiments with OpenArray™ plates”). 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.

- Vortex, then briefly centrifuge the plate that contains the completed preamplification sample reactions.
- Remove the adhesive film from the plate.

3. Prepare a 1:10 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 μ L).
 - b. Add the appropriate volume of Nuclease-free Water to each sample and control well (for example, 18 μ L).
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 respiratory tract microbiota profiling experiments with OpenArray™ plates”).

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



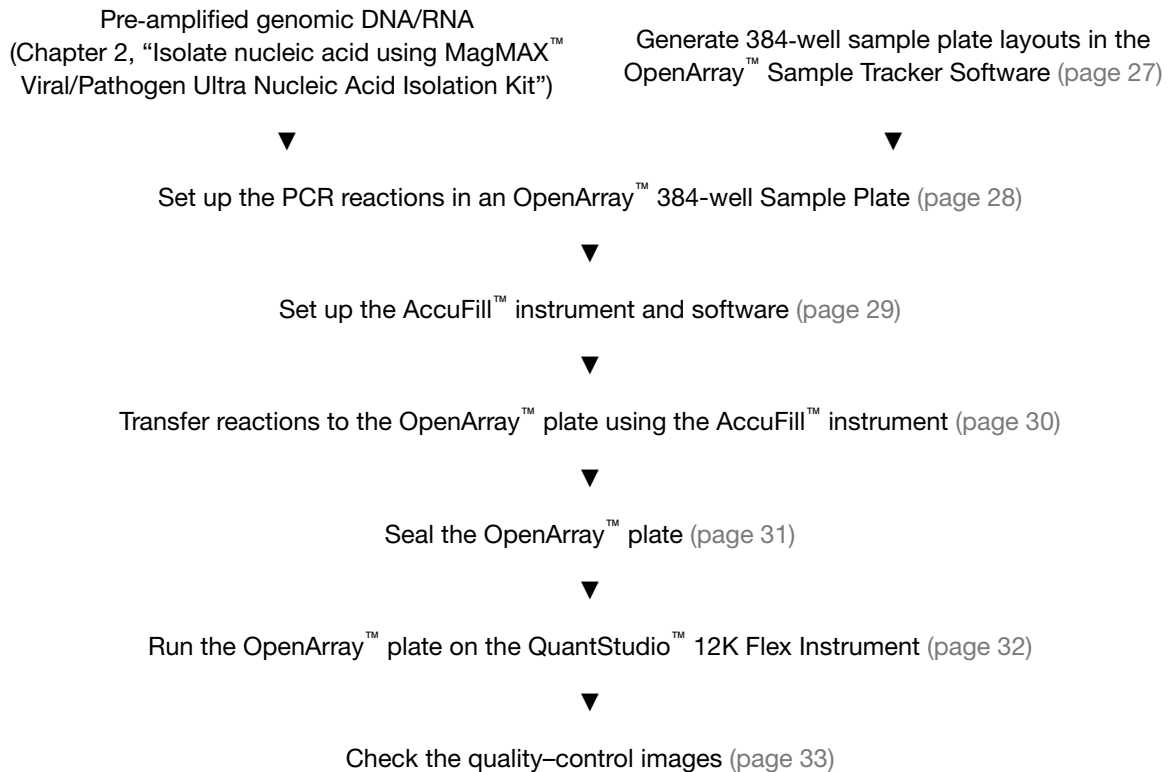
Prepare and run respiratory tract microbiota profiling experiments with OpenArray™ plates

For required materials, see “Materials required for OpenArray™ plate workflow” on page 13.

This chapter contains brief procedures. For detailed procedures, see the following documentation.

Document	Pub. No.
<i>QuantStudio™ 12K Flex Real-Time PCR System: OpenArray™ Experiments User Guide</i>	4470935
<i>OpenArray™ Sample Tracker Software Quick Reference</i>	4460657
<i>OpenArray™ AccuFill™ System User Guide</i>	4456986

Workflow: Microbiota profiling experiments with OpenArray™ plates



One-time procedures

Set up default folders and software preferences

This procedure simplifies the file locations used in the AccuFill™ software.

Set up the default file locations and preferences before using the OpenArray™ AccuFill™ system for the first time. You must be logged in as an administrator.

1. Create the following four folders in a convenient location on the same computer drive as the AccuFill™ software:
 - **TPF Files**
 - **Sample Tracker 96-well Input**
 - **Sample Tracker 384-well CSV Files**
 - **Loaded TPF Files**

2. (Optional) Copy a template file into the OpenArray™ Sample Tracker Software folder.
 - Navigate to this folder on your computer: <drive>:\Program Files (x86)\Applied Biosystems\OpenArray Sample Tracker\examples.
 - Copy the 96-Well Sample Plate 1.csv template file, which is provided with the OpenArray™ Sample Tracker Software.
 - Paste the template file into the **Sample Tracker 96-well Input** folder.
3. In the OpenArray™ Sample Tracker Software, select **View ▶ Preferences**, then enter the following preferences:

Field	Selection
Experiment Type	Gene Expression
OpenArray™ Plate	Select the OpenArray™ format that will be run most often, such as Gene Expression – 112.
Pipettor	Fixed or Adjustable tip spacing
Import Data Directory	Sample Tracker 96-well Input
Export Data Directory	Sample Tracker 384-well CSV Files

4. In the AccuFill™ software, select **Instrument ▶ Edit Preferences**, then:
 - a. Select **Require Sample Integration**.
 - b. Select the folders indicated in this table:

AccuFill™ folder	Default folder	Folder contents
OpenArray™ Plate File Input Folder	TPF Files	TPF files for the OpenArray™ plates, with assay name and location
Sample Plate File Folder	Sample Tracker 384-well CSV Files	CSV 384-well sample plate layout files
Loaded OpenArray™ Plate File Folder	Loaded TPF Files	Integrated TPF files generated during processing with the AccuFill™ software.

5. In the QuantStudio™ 12K Flex Software, select **Tools ▶ Preferences ▶ OpenArray**, then select the **Loaded TPF Files** folder for the software **Setup Folder**.

Note: If the QuantStudio™ 12K Flex Software is not on the same computer as the AccuFill™ software, transfer the loaded TPF files to the computer running the QuantStudio™ 12K Flex Software.

Obtain TPF files


Go to thermofisher.com/OA-platefiles to obtain the TPF files for the TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate.

Set up the optimized folder locations and software preferences before downloading TPF files to your computer. See “Set up default folders and software preferences” on page 25.

Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software

Before generating 384-well sample plate layouts, see “One-time procedures” on page 25 to complete the following tasks:

- Set up optimized folder locations and software preferences.
 - Download the TPF files for the OpenArray™ plates into the TPF Files folder.
1. Using a spreadsheet program, create a 96-well sample CSV file.
 - a. Navigate to the following folder, then open the *96-Well Sample Plate 1.csv* template that is provided with the OpenArray™ Sample Tracker Software.

```
<drive>:\Program Files (x86)\Applied Biosystems\OpenArray Sample Tracker\examples
```
 - b. **Save As** the template as a new 96-well sample CSV file. Save your 96-well sample CSV file in the **Sample Tracker 96-well Input** folder.
 - c. Enter or copy the sample names into your 96-well sample CSV file.
 2. Open the OpenArray™ Sample Tracker Software.
 3. In the **Properties** screen, select **Gene Expression** for **Experiment Type**, then select the appropriate settings for **OpenArray™ Plate** and **Pipettor**.
 4. In the **Samples** screen, click  **Import**, then select and import your 96-well sample CSV file that you created in step 1.
 5. In the **Sample Mapping** screen, confirm that the samples for a single OpenArray™ plate are assigned to one color.

Note: If necessary, correct the **OpenArray™ Plate** and **Pipettor** settings in the **Properties** screen.

6. In the **Sample Mapping** screen, click the **384-Well Plate** tab, then click **Export** ▶ **Export *.csv**.
7. Select **384-Well Plate (for AccuFill)**, enter a file name, then save the exported file.

Plate layouts for the 384-well sample plates are saved to individual CSV files in the **Sample Tracker 384-well CSV Files** folder.

Set up the PCR reactions in an OpenArray™ 384-well Sample Plate

IMPORTANT! The 4 × 12 area(s) of the OpenArray™ 384-well Sample Plate being filled must match the area(s) designated in the OpenArray™ Sample Tracker Software for that set of samples.

1. Remove an OpenArray™ plate from the freezer and set it aside. Allow it to come to room temperature in its unopened sleeve (~15 minutes).
The OpenArray™ plate must be completely thawed before transferring reactions to it from the OpenArray™ 384-well Sample Plate created in this section.
2. Gently swirl the contents of the TaqMan™ OpenArray™ Real-Time PCR Master Mix to thoroughly mix. Do not invert the bottle.
3. Following the plate layout designated in the OpenArray™ Sample Tracker Software, add master mix, then DNA samples, to the wells of an OpenArray™ 384-well Sample Plate.
(Optional) Use the TaqMan™ Respiratory Tract Microbiota Amplification Control in place of diluted preamplified sample, as a positive amplification control sample.

Component	Volume per well	Volume per sample ^[1]
TaqMan™ OpenArray™ Real-Time PCR Master Mix	2.5 µL	5.5 µL
Diluted preamplified sample	2.5 µL	5.5 µL
Total reaction volume	5.0 µL	11.0 µL

^[1] Volume includes 10% overage.

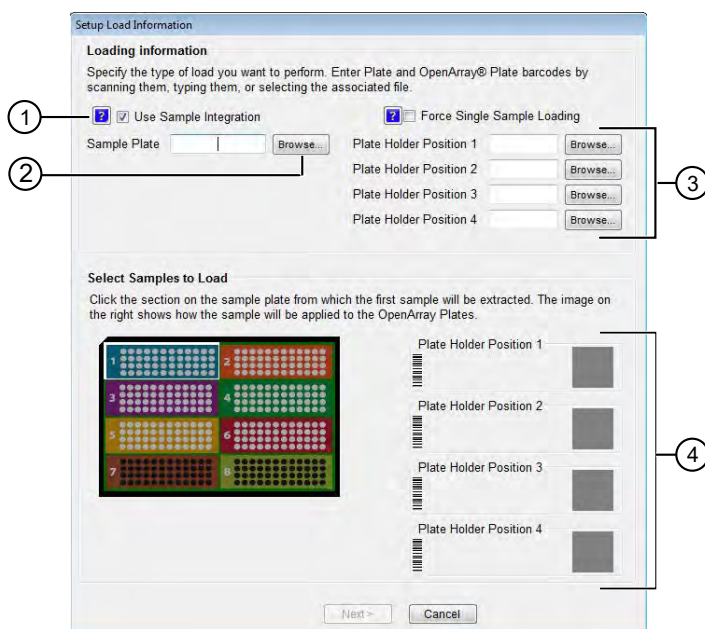
4. Thoroughly mix each PCR reaction by pipetting up and down or by using the "mix" function on a multi-channel pipette.
5. Seal the OpenArray™ 384-well Sample Plate with an aluminum foil seal, remove the foil flap, then mark the edges of the filled 4 × 12 area with a pen.
6. Centrifuge the plate at 1,200 × g for 1 minute.
7. Score the foil along the lines that were marked before centrifuging.
Do not remove the foil from the scored area at this time.

If you make a sample layout error before the AccuFill™ procedure – Repeat “Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software” on page 27 with a corrected 96-well sample CSV file.

Set up the AccuFill™ instrument and software

IMPORTANT! Do not use OpenArray™ AccuFill™ System Tips that exceed the expiration date (shown on the outer box that contains the tip trays).

1. In the OpenArray™ AccuFill™ software, click **Setup and Load**.
The **Setup Load Information** window appears.



- ① **Use Sample Integration** checkbox; select to integrate TPF files and the 384-well sample plate CSV file.
 - ② **Browse** button; click to locate and select the 384-well sample plate CSV file. The button is displayed only if **Use Sample Integration** is selected.
 - ③ **Browse** buttons; click to locate and select the TPF files for the OpenArray™ plates that will be placed in the corresponding **Plate Holder Position** on the deck of the AccuFill™ instrument. The buttons are displayed only if **Use Sample Integration** is selected.
 - ④ **Plate Holder Position** corresponding to the position of the OpenArray™ plate on the deck of the AccuFill™ instrument.
2. Configure the **Loading Information** pane for sample integration using the 384-well sample plate CSV file and TPF files.
 - a. In the **Loading Information** pane (top section of the window), ensure that the **Use Sample Integration** checkbox is selected.
 - b. Click **Browse** to the right of the **Sample Plate** field, then select the 384-well sample plate CSV file that you generated with the OpenArray™ Sample Tracker Software in the Sample Tracker 384-well CSV Files folder.
 - c. Click **Browse** to the right of the **Plate Holder Position** of the OpenArray™ plate, then select the TPF file for the OpenArray™ plate in the TPF Files folder.

3. In the **Select Samples to Load** pane (bottom section of the window), click the corresponding 4 × 12 area of the 384-well sample plate image, then click **Next**.
The **Setup Deck** window is displayed.
4. In the AccuFill™ instrument, ensure that:
 - Tip boxes and tips are loaded as shown in the **Setup Deck** window.
 - The lids are removed from the tip boxes.
 - The waste bin in the instrument is emptied.
5. In the **Setup Deck** window, confirm that the deck is ready:
 - Select **The tips are configured as shown above**.
 - Select **The Waste Bin is empty**.

Transfer reactions to the OpenArray™ plate using the AccuFill™ instrument

IMPORTANT! Ensure that the OpenArray™ plate is thawed and that the entire plate is at room temperature.

1. Prepare the items needed to seal the loaded OpenArray™ plate (next section).

Note: The OpenArray™ plate must be sealed promptly after being loaded with the reactions, as described here.

- Ensure that the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0 is ready.
 - Gather and remove from their packaging the following: an OpenArray™ Lid, plug, syringe with OpenArray™ Immersion Fluid, and syringe tip.
 - Attach the syringe tip to the syringe, carefully push some of the fluid through the tip to remove air bubbles, then lay the syringe aside.
2. Load the OpenArray™ plate and the OpenArray™ 384-well Sample Plate into the AccuFill™ instrument.
 - **OpenArray™ plate**—Remove the plate from its sleeve, then place the plate in the appropriate plate holder position in the instrument.
Ensure that the barcode on the OpenArray™ plate is facing left and the serial number is facing right.
 - **OpenArray™ 384-well Sample Plate**—Place the 384-well sample plate onto the deck of the instrument, then use forceps to peel the foil from the filled area of the plate.
 3. Close the door of the AccuFill™ instrument.
 4. In the AccuFill™ software **Setup Deck** window, select the following confirmations:
 - **The OpenArray Plate is in the Plate Holder**
 - **Remove foil from the highlighted section of the Sample Plate**

5. Click **Load**.
6. As soon as the **Remove OpenArray Plate** window appears, open the instrument door and remove the loaded OpenArray™ plate.
7. Proceed immediately to seal the OpenArray™ plate (next section).

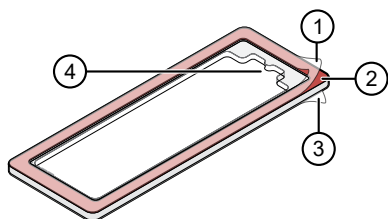
Note: For best results, seal the OpenArray™ plate within 90 seconds of completion of loading to prevent evaporation.

Seal the OpenArray™ plate

IMPORTANT! Throughout this procedure, handle the OpenArray™ plate and the OpenArray™ Case only by the edges.

Note: The OpenArray™ Case consists of the sealed OpenArray™ plate and the OpenArray™ Lid.

1. Place the newly loaded OpenArray™ plate in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.
Ensure that the barcode is facing left and the serial number is facing right.
2. From the OpenArray™ Lid, remove the clear protective film from the *inside* of the lid and the red adhesive-protective strip from around the edge of the lid.



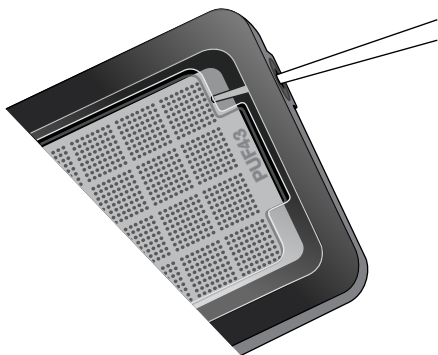
OpenArray™ Lid

- ① Protective film on inside of the lid (remove before *sealing*)
 - ② Red adhesive-protective strip (remove before *sealing*)
 - ③ Protective film on the outside of the lid (remove before *running*)
 - ④ Notched end (align with serial number on plate)
3. Seat the lid on the OpenArray™ plate with the lid adhesive against the plate.
 4. Engage the press mechanism until the green flashing light changes to a steady green light (approximately 20 seconds).
 5. Disengage the press and remove the OpenArray™ Case.

6. While holding the case by its edges, insert the prepared syringe tip into the port in the case, then carefully inject OpenArray™ Immersion Fluid until the case is filled.

Note:

- Minimize creation of air bubbles when you dispense the fluid.
- Leave a small bubble at the fill point to prevent fluid leaks during the instrument run.



The syringe tip must be in front of the array when filling the case with immersion fluid.

7. While holding the case *vertically*, remove the syringe tip, insert the screw end of the OpenArray™ plug into the port of the case, then rotate clockwise until the black handle breaks off.



IMPORTANT! To avoid leaking of immersion fluid, hold the case *vertically* and rotate the plug slowly.


If the plug handle breaks off prematurely, use a Phillips #0 screwdriver to complete this step.

8. If needed, clean the case with a laboratory wipe that has been thoroughly sprayed with ethanol, then dry the case with a clean laboratory wipe.

Run the OpenArray™ plate on the QuantStudio™ 12K Flex Instrument

You can run up to four OpenArray™ plates at one time on the QuantStudio™ 12K Flex Instrument.

1. On the QuantStudio™ 12K Flex Instrument touchscreen, touch  to extend the instrument tray arm.
2. Remove the clear protective film from the outside of the OpenArray™ case (sealed plate + lid).
3. Place the OpenArray™ case on the tray arm plate adapter.
 - Support the case from underneath the tray arm to prevent the case from slipping through the adapter.
 - Ensure that the plate barcode and serial number are facing the front of the instrument.
4. Touch  to retract the instrument tray arm.

5. In the  **Home** screen of the QuantStudio™ 12K Flex Software, in the **Run** pane, click **OpenArray**.
6. In the **Select Instrument** pane, select your instrument.
7. Click **Get Plate IDs** to import the barcode of the OpenArray™ plate.
Once the OpenArray™ serial number appears, the loaded TPF file corresponding to the plate should appear in the **Setup File** field.
If the TPF file does not appear, click **Browse**, then select the correct loaded TPF file from the **Loaded TPF** folder.
8. (Optional) Click **Browse** to change the **Experiment File Location**.
9. (Optional) Change the software-determined **Experiment File Name**.
10. Click **Start Run**.

Note: The instrument pauses at 41 or 42 seconds prior to the end of the run. Wait for the system to complete the run before opening the EDS file.

11. Transfer the EDS file from the instrument to an accessible location for analysis.
12. Check the QC images for loading issues or leaks.

Check the quality-control images

Check the quality-control (QC) images before analysis. Images can be viewed using ImageJ, an open-source software available from the NIH at imagej.nih.gov/ig. For additional information, see Appendix A, “Troubleshooting”.

1. In the QuantStudio™ 12K Flex Software  **Export** screen, click **Browse**, then create a uniquely-named folder for the QC images export.

IMPORTANT! Create a new folder for images each time. Exporting a second run to the same folder overwrites the images.

2. Click **Export QC Images** at the bottom of the screen.
3. View the following ROX™ image to check for loading quality issues:
 - POST-READ_CHANNEL_4.tiff
4. Check the following spotfinding images for leaks or other displaced sample issues:
 - s02_c001_t03_p0001_m1_x2_e1_cp#_spotfind.tiff
 - s02_c040_t03_p0001_m1_x2_e1_cp#_spotfind.tiff


Note: The “cp#” in the image file name refers to array positions 1 through 4 within the instrument.

5. If a problem is found, view the following pre-run spotfinding image to determine whether the issue existed before cycling:
 - s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff
6. View the following FAM™ images to check for fluorescent abnormalities and to confirm any problem seen in the spotfinding images:
 - STAGE2_CYCLE1_CHANNEL_1.tiff
 - STAGE2_CYCLE40_CHANNEL_1.tiff
7. Note any abnormalities found, as well as all other potentially relevant information related to the setup of the run.

5

Analyze data


Select analysis software

Software	Analysis option	
	C _{rt}	QC metrics
Relative Quantification Application  (recommended) ^[1] Access the application at thermofisher.com/connect .	✓	✓
QuantStudio™ 12K Flex Software	✓	✓

^[1] To perform data analysis using the application, you must export your data. For detailed instructions about exporting data, see “Export data (if necessary)” on page 35.

Export data (if necessary)

Export your data to review it using the pivot table feature of a spreadsheet program.



1. Open an EDS file in the QuantStudio™ 12K Flex Software.
2. In the **Experiment Menu** pane, click  **Export**.
3. Click **Load Export Set** (bottom of the screen), select **GE_export_setting**, then click **OK**.
4. Select **.xlsx** from the **File Type** dropdown list (top-right of the screen).
5. (*Optional*) Perform any of the following actions to customize the file export.
 - Click **Browse** to select a new **Export File Location**.
 - Enter a new file name in the **Export File Name** text field.
 - Click the **Results** tab, then select the content to export.
6. Click **Start Export** (bottom of the screen).
If **Open file(s) when export is complete** is selected, then the file automatically opens. If the option is not selected, navigate to and open the exported XLSX file.

Review results

Note: These guidelines apply to results from experiments that included three or more technical replicates.

1. In the analysis settings of the software, choose 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 Relative Quantification Application :
 - a. Click  (**Analysis Settings**).
 - b. In the **C_q Settings** tab, select **Use C_{rt}** .
 - c. Click **Finish**.
- In QuantStudio™ 12K Flex 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}	$C_{rt} > 28$
Amp Score	<p>Amp Score < 1.2</p> <p>The following assays have a cut-off value of < 1.1</p> <ul style="list-style-type: none"> • RNase P (RPPH1) • Human Enterovirus (EV_pan)
C_q Confidence	<p>C_q Conf < 0.7</p> <p>Note: The following assays have a cut-off value of < 0.5</p> <ul style="list-style-type: none"> • RNase P (RPPH1) • Bordetella holmesii (B. holmesii)

Note:

- We encourage testing and establishing your own C_{rt} cut-off value for each assay to achieve high sensitivity and specificity.
- Through-holes with unexpected C_{rt} values can also be identified by reviewing the Amplification Plot (see “Troubleshoot unexpected C_{rt} values” on page 40).

4. Note replicates with mean $C_{rt} \leq 25$ and a standard deviation > 0.5 . The data from these through-holes might require further review.
5. Ensure that at least half of the replicates amplified adequately and pass your review specifications.
6. Use your preferred method to analyze the data.

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.

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

Species-specific assay	Considerations for data analysis
Flu A assays	<ul style="list-style-type: none"> • 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.
Bordetella assays	<ul style="list-style-type: none"> • The Bordetella assay detects <i>B. pertussis</i>, <i>B. bronchiseptica</i>, and <i>B. parapertussis</i> strains. Strain-specific assays for <i>B. pertussis</i> and <i>B. holmesii</i> are also available. • Most samples that are positive for the <i>B. pertussis</i> assay are also positive with the Bordetella assay.
RSV assays	<ul style="list-style-type: none"> • Samples that are positive for the RSV_A assay may be detected at a lower efficiency (a difference of several C_{rt} values) by the RSV_B assay.
Enterovirus (EV) and rhinovirus (RV) assays	<ul style="list-style-type: none"> • 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.

(continued)

Species-specific assay	Considerations for data analysis
Bacterial and HHV assays	<ul style="list-style-type: none"> It is not unusual to detect <i>M. catarrhalis</i>, <i>H. influenzae</i>, <i>K. pneumoniae</i>, <i>S. pneumoniae</i>, and <i>S. aureus</i> 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	18–20
TaqMan™ Universal Extraction Control Organism (<i>B. atrophaeus</i>)	21–23
TaqMan™ Respiratory Tract Microbiota Amplification Control	18–21

Fields for reviewing results with pivot tables

To review results using the pivot table feature of a spreadsheet program, you can use the following settings.

Note: For the "Average of" and "StdDev of" summarizations, use the appropriate source field (C_{rt} , **Amp Score**, or **C_q Conf**), then choose the calculation type.

Area of pivot table	Fields to add	
	Target-oriented view	Sample-oriented view
Filters	—	Sample Name ^[1]
Columns	Sample Name	—
Rows	Target Name	Target Name
Values	Average of C_{rt}	Average of C_{rt}
	StdDev of C_{rt} ^[2]	StdDev of C_{rt} ^[2]
	Count of C_{rt}	Count of C_{rt}

(continued)

Area of pivot table	Fields to add	
	Target-oriented view	Sample-oriented view
Values	—	Average of Amp Score
	—	Average of C _q Conf

^[1] To see individual sample results, select the sample from the dropdown list next to the **Sample Name** header.

^[2] A **Values** field will automatically appear in the **Column Labels** area.

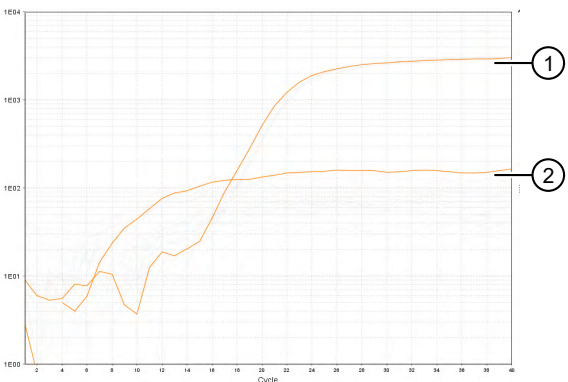


Troubleshooting

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 μ L.
		Reduce the input volume of starting sample to 200 μ L.
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.


Troubleshoot unexpected C_{rt} values

Observation	Possible cause	Recommended action	
Unexpected C_{rt} values in the amplification plot  <p>① Expected C_{rt} value (noted in most replicates) ② Unexpected C_{rt} value (too low)</p>	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.	
		Unexpectedly high C_{rt} values (25–28) – Sporadic amplification	Dilute the samples, then repeat the experiment.
			Review amplification curves, Amp Score, and C_q Confidence values.
			Compare replicates, if available.
		Repeat the experiment.	

Troubleshoot with cycling and imaging run images (QC images)

Many problems with OpenArray™ results can be diagnosed by examining the quality control (QC) images taken at various points during a cycling/imaging run.

The QC images are fluorescent or reflected light images taken before, during, and after cycling. They may require adjustment to make image features visible. To view the images, we recommend that you install the free software program ImageJ, which allows you to easily manipulate the images in ways that other image viewers cannot.

1. In the QuantStudio™ 12K Flex Software **Export** screen :
 - a. Click **Browse** to select a uniquely-named folder for the QC images export.
 - b. Click **Export QC Images** (bottom of screen).

IMPORTANT! Select a new folder for images each time; exporting a second run to the same folder overwrites the images.


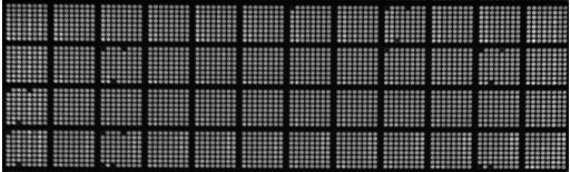
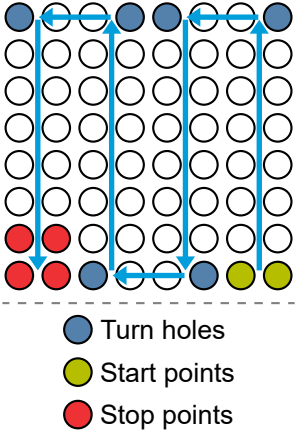
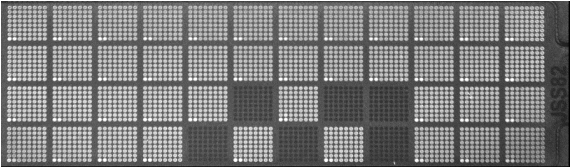
2. Use ImageJ to view the images of interest.

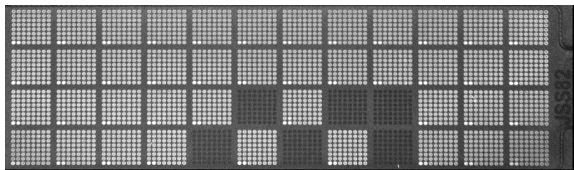
To...	View image...	Image description
Confirm the identity of images within a folder	BARCODE IMAGE.tiff	Reflected light image of the entire OpenArray™ plate
Evaluate the loading quality	PRE-READ_CHANNEL_4.tiff POST-READ_CHANNEL_4.tiff	Pre- and post-ROX™ dye images
Check for existing contamination on the case and/or heated cover	s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff ^[1]	Pre-run reflected light spotfinding image (used by the software to determine the location of the holes)
Identify potential leaks or other contamination	s02_c001_t03_p0001_m1_x2_e1_cp#_spotfind.tiff ^[1]	Mid-run reflected light spotfinding image
	s02_c040_t03_p0001_m1_x2_e1_cp#_spotfind.tiff ^[1]	Post-run reflected light spotfinding image
Look at patterns in the fluorescent data (for example, gradients)	STAGEx_CYCLEy_CHANNEL_1.tiff	FAM™ images at a particular cycle (y) of a particular stage (x) of the run.

^[1] The “cp#” in the image file name refers to the array position (1–4) within the QuantStudio™ 12K Flex Real-Time PCR Instrument.

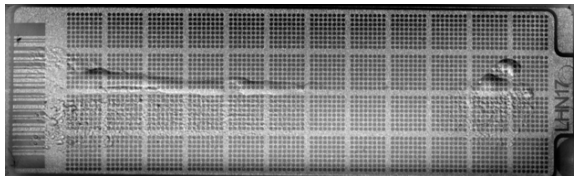
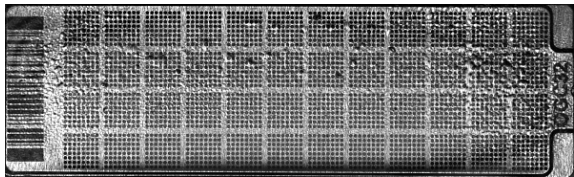
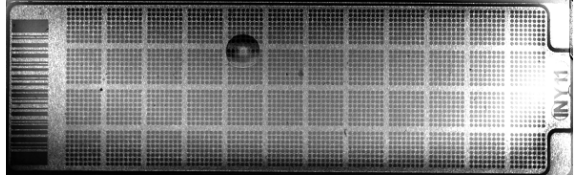
3. (Optional) Adjust the images for brightness and/or contrast to make image features visible.
 - a. Open the image in ImageJ.
 - b. Select **Image ▶ Adjust Brightness/Contrast** (or press **Ctrl+Shift+C**).
 - c. Click **Auto** or adjust the sliders until the features of interest in the image are visible.

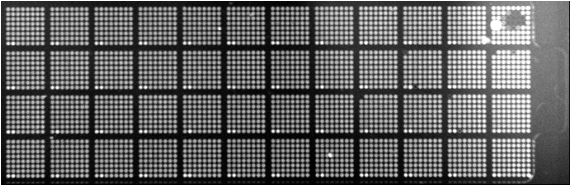
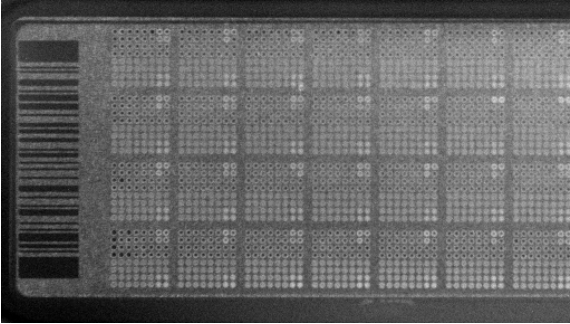
AccuFill™ instrument plate loading errors

Observation	Possible cause	Recommended action
There are empty through-holes 	Insufficient sample was added to the 384-well Sample Plate.	Use proper pipetting techniques. Ensure that there are no air bubbles in the pipette tips after sample aspiration.
	Reaction mix (sample + master mix) is not at the bottom of the 384-well Sample Plate.	Centrifuge the plate at 1,200 × g for 60 seconds.
Turn-holes are repeatedly missed 	AccuFill™ instrument is aligned too far to the left or to the right. Systematic loading problems can occur with the AccuFill™ instrument, which indicates a need for service. For example, when turn-holes are repeatedly missed across multiple subarrays, service is required. Turn-holes are where the AccuFill™ instrument changes direction during sample loading.	Contact your local field service engineer.
		
Entire subarrays are missing 	The sample/master mix was not added to particular wells in the 384-well Sample Plate.	Visually inspect the plate to ensure that the wells have sample/master mix.
	Stuck tip mandrel on AccuFill™ instrument may need cleaning.	Contact your local field service engineer.

Observation	Possible cause	Recommended action
<p>Entire subarrays are missing</p>  <p>(continued)</p>	<p>Pipette tip was not loaded on mandrel.</p>	<p>Contact your local field service engineer for frequent occurrences (infrequent occurrences can be due to a poorly molded tip).</p>

OpenArray™ plate assembly and handling errors

Observation	Possible cause	Recommended action
<p>Case leaks and bubbles inside the case</p> 	<p>Plate press was not engaged for at least 20 seconds.</p>	<p>Fully engage the plate press for at least 20 seconds.</p>
	<p>Damaged lid adhesive.</p>	<p>Remove the liner and visually inspect the lid adhesives for defects. Ensure that adhesive is not damaged or warped.</p>
	<p>Damaged fill port screw gasket.</p>	<p>Visually inspect the screw to ensure that the orange gasket is present and not damaged.</p>
<p>Improper sealing of the OpenArray™ plate in the OpenArray™ Case can lead to immersion fluid leaks or bubble formation inside the case, leading to uneven heating and imaging throughout PCR and to poor quality data.</p> <p>The images above are examples of OpenArray™ plates that have been affected by immersion fluid leaks. The images show where leaked fluid has condensed on the underside of the heated cover</p>	<p>Damaged fill port screw assembly. Breaks off too easily.</p>	<p>The screw may be mis-threaded: unscrew it and use a new screw assembly.</p>
	<p>Oily lid or case from immersion fluid overflow.</p>	<p>Wipe off excess overflow of immersion fluid from the lid, case bottom, and crevices with 70% isopropyl alcohol, using a lint-free cloth (the cloth included with the OpenArray™ plate is acceptable).</p>
	<p>Immersion fluid was exposed to air for too long.</p>	<p>Do not remove the immersion fluid syringe cap or draw air bubbles into the syringe until you are ready to load.</p>

Observation	Possible cause	Recommended action
<p>windows and obscured the view of the through-holes.</p> <p>The best image in which to detect leaks is the s02_c001_t03_p0001_m1_x2_e1_cp#_spotfind.tiff image. This image is taken at the start of cycling, which is where most leaks occur. See “Troubleshoot with cycling and imaging run images (QC images)” on page 41.</p>	<p>Too large of a bubble inside the OpenArray™ Case after sealing.</p>	<p>Minimize the size of the bubble by tilting the OpenArray™ Case so that the fill port is at the highest point. Slowly fill the case with immersion fluid until only a small air bubble remains. Attach the screw and wipe off any excess oil that may have spilled onto the case.</p>
	<p>Damaged plate press, leading to uneven pressure.</p>	<p>Contact your field service engineer if you suspect that your plate press may be damaged.</p>
<p>Sample blow-out during the addition of immersion fluid</p> 	<p>The reactions in A12 were compromised during the addition of immersion fluid. Injecting the immersion fluid too quickly can purge the sample out of the through-holes near the fill port. Often this is caused by the user not purging the syringe slightly before use.</p>	<p>Dispense a small amount of immersion fluid onto a paper towel before use to ensure smooth operation of the syringe.</p>
<p>Evaporation of reaction mixture in through-holes</p> 	<p>Too much time elapsed before the plate was sealed with lid and immersion fluid. In this example, the top half of each subarray was intentionally left open to the environment to demonstrate the effect of evaporation. “Donuts” are a result of the evaporated fluid in the though-holes.</p>	<p>Add immersion fluid as soon as the case is removed from the plate press to minimize the likelihood of evaporation, then seal the case with the lid.</p>



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
<i>Isolation of Nucleic Acid for Respiratory Tract Microbiota Profiling Experiments Quick Reference</i>	MAN00018526
<i>Respiratory Tract Microbiota Profiling Experiments using OpenArray™ Quick Reference</i>	MAN0018529
<i>TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate Product Information Sheet</i>	MAN0018631
<i>TaqMan™ Respiratory Tract Microbiota Amplification Control Product Information Sheet</i>	MAN0018533
<i>TaqMan™ Universal Extraction Control Organism (B. atrophaeus) Product Information Sheet</i>	MAN0018535
<i>TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control Product Information Sheet</i>	MAN0018534
<i>QuantStudio™ 12K Flex Real-Time PCR System: OpenArray™ Experiments User Guide</i>	4470935
<i>QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide</i>	4470689
<i>Thermo Scientific™ KingFisher™ Flex User Manual</i>	N07669
<i>OpenArray™ Sample Tracker Software Quick Reference</i>	4460657
<i>OpenArray™ AccuFill™ System User Guide</i>	4456986
<i>TaqPath™ 1-Step RT-qPCR Master Mix, CG User Guide</i>	MAN0007959

Symbols that may be displayed on the instrument, in the software, or in this guide

Symbol	Description	Symbol	Description
	MANUFACTURER		DATE OF MANUFACTURE
	CATALOG NUMBER		SERIAL NUMBER
	CONSULT INSTRUCTIONS FOR USE ^[1]		CAUTION ^[1]

^[1] Appendix C, “Documentation and support”



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.

