Urinary Tract Microbiota Profiling Experiments

APPLICATION GUIDE

TaqMan™ Assays for urinary tract microbiota profiling experiments in TaqMan™ OpenArray™ Plate-format

for use with:

TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate

Custom TaqMan™ OpenArray™ Plates

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

Catalog Numbers A39900

Publication Number MAN0017750

Revision E

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

Revision history: MAN0017750 E (English)

Revision	Date	Description
E	16 September 2024	• Vortex instructions were updated ("Set up the PCR reactions in an OpenArray™ 384-well Sample Plate (OpenArray™ AccuFill™ Software v2.0)" on page 35). • Sealing instructions were updated ("Seal the OpenArray™ Plate" on page 42). • Minor verbiage updates throughout document.
D.0	4 January 2022	• The target organism name of *Enterobacter aerogenes* was updated to *Klebsiella aerogenes*. • TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*) was added as an optional control. • For the DNA isolation procedure, the MagMAX™ DNA Multi-Sample Ultra Kit was replaced with the MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit (Cat. No. A42356). • The KingFisher™ script for download was updated to **MVP_Ultra_Flex**. • The guidelines were updated to include the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head and 96 deep-well heating block and to recommend a new MicroAmp™ Clear Adhesive Film for each step of the procedure ("Procedural guidelines" on page 16). • The following updates were made to the list of required materials for the OpenArray™ Plate workflow: – Clear plates were added as an option for the OpenArray™ 384-well Sample Plates. – The Biomek™ Seal and Sample Foil Lids were changed to an optional material. – A sharp edge, a blade, or a scalpel was added. – Nuclease-free water was added. – The ethanol was updated to 100% molecular grade ethanol. • The location of the sample layout file was corrected for OpenArray™ Sample Tracker Software (applies to OpenArray™ AccuFill™ Software v1.2 only). • The note to recommend a multichannel pipette when concentrating the sample during DNA isolation was removed. • The recommendations for remaining supernatant when concentrating the sample during DNA isolation were updated. A remaining volume of 30–100 µL is acceptable. It is more important to not disrupt the pellet than to remove all of the supernatant. • The Biomek™ Seal and Sample Foil Lids were changed to an optional material. • The email address for information about amplification controls was updated to **GeneArtSupport@thermofisher.com**. • A note was added to recommend a pipetting overage when setting up the PCR reactions. • The speed requirement of the centrifuge for the OpenArray™ Plate workflow was updated. • The centrifuge speed was updated when preparing PCR reactions in an OpenArray™ 384-well Sample Plate and troubleshooting empty through-holes. • Separate chapters for preparing OpenArray™ Plates with OpenArray™ AccuFill™ Software v1.2 and OpenArray™ AccuFill™ Software v2.0 were added. • A separate chapter for sealing and running OpenArray™ Plates was added. • The instructions to export the data were corrected to include a step to analyze the data before they are exported.
C.0	29 March 2021	Updated information for TaqMan™ Universal DNA Spike In Control.
B.0	9 August 2018	• Added information for the TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate. • Added references for additional user documentation. • Updated DNA isolation protocol per V&V testing.
A.0	25 March 2018	New document.

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

Contents

1 Introduction and workflow overview

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

This guide describes the OpenArray™ Plate high-throughput, sample-to-result workflow for urinary tract microbiota profiling. The workflow uses the following components:

- MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit for DNA isolation from urine samples
- OpenArray™ Plates with TaqMan™ Assays for urinary tract microbiota profiling
- QuantStudio™ 12K Flex Instrument with OpenArray™ block (QuantStudio™ 12K Flex OpenArray™ AccuFill™ System)

Urinary tract microbiota profiling

Microorganism-specific TaqMan™ Assays offer a rapid and accurate approach to investigate and monitor urinary tract microbiome composition and dynamics.

We offer a collection of qualified TaqMan™ Assays that are optimized for detection of urinary tract microbes (see "TaqMan™ Assays for urinary tract microbiota profiling" on page 10). The TaqMan™ Assay design and their target sequences have undergone rigorous bioinformatics selection and analysis to allow maximum strain coverage and minimal off-target cross-reactivity. Qualified TaqMan™ Assays for urinary tract microbiota profiling demonstrate accurate, reproducible performance in multiple rounds of testing for sensitivity and specificity. The assays perform well with DNA isolated from urine samples using optimized MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit protocols.

Additional TaqMan™ Assays for microbial targets are available from our predesigned assay collection. For Custom TaqMan™ Assays contact **QuantStudioFrontDesk@thermofisher.com**.

Workflow

TaqMan™ urinary tract microbiota profiling experiments

Compatible sample collection and storage (page 14)

Isolate DNA from urine research samples using the MagMAX™ Viral/ Pathogen Ultra Nucleic Acid Isolation Kit (page 14)

Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v1.2 (page 21) **OR Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0** (page 32)

Seal and run the OpenArray™ Plates (page 42)

Export and review urinary tract microbiota profiling data (page 47)

2 Background and tools for assay selection

TaqMan™ Assays

TaqMan™ Assays for urinary tract microbiota profiling consist of a pair of unlabeled PCR primers and a TaqMan™ probe with a FAM™ dye label on the 5' end and minor groove binder (MGB) and nonfluorescent quencher (NFQ) on the 3' end.

For more information about real-time PCR and TaqMan™ Assays, go to **thermofisher.com/qpcreducation**.

TaqMan™ Assays for urinary tract microbiota profiling

All of the TaqMan™ Assays for urinary tract microbiota profiling, including the controls, are included in the TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate. The assays can also be configured with Custom TaqMan™ OpenArray™ Plate (see "TaqMan™ OpenArray™ Plate products and formats" on page 12). For optional reference and controls, see "Optional controls" on page 11.

For more information about available TaqMan™ Assays for urinary tract microbiota profiling, go to **thermofisher.com/utm**.

Table 1 TaqMan™ Assays for urinary tract microbiota profiling

Assay ID	Classification	Target organism name
Ba04932084_s1	Bacteria	*Acinetobacter baumannii*
Ba04932088_s1	Bacteria	*Citrobacter freundii*
Ba04932087_s1	Bacteria	*Enterobacter cloacae*
Ba04646247_s1	Bacteria	*Enterococcus faecalis*
Ba04932086_s1	Bacteria	*Enterococcus faecium*
Ba04646242_s1	Bacteria	*Escherichia coli*
Ba04932080_s1	Bacteria	*Klebsiella aerogenes*
Ba04932079_s1	Bacteria	*Klebsiella oxytoca*
Ba04932083_s1	Bacteria	*Klebsiella pneumoniae*
Ba04932078_s1	Bacteria	*Morganella morganii*
Ba04932076_s1	Bacteria	*Proteus mirabilis*
Ba04932082_s1	Bacteria	*Proteus vulgaris*
Ba04932077_s1	Bacteria	*Providencia stuartii*
Ba04932081_s1	Bacteria	*Pseudomonas aeruginosa*
Ba04932085_s1	Bacteria	*Staphylococcus saprophyticus*
Ba04646276_s1	Bacteria	*Streptococcus agalactiae*
Fn04646233_s1	Yeast	*Candida albicans*
***(Optional)* Control assays**[1]		
Hs04930436_g1	Control	Human RNase P RPPH1 gene[2]
Ac00010014_a1	Control	Xeno™[3]

[1] Included in the TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate.
[2] Use to assess sample adequacy.
[3] Use to control for nucleic acid recovery in sample preparation process.

Optional controls

(Optional) TaqMan™ Universal DNA Spike In Control

The TaqMan™ Universal DNA Spike In Control (Cat. No. A39175) is an exogenous Xeno™ DNA process control that can be used to monitor the recovery efficiency for the DNA extraction and purification process. The control also indicates the presence of PCR inhibitors in molecular detection workflows. This control is of particular importance when working with urine samples that can have a higher frequency of inhibition.

This product contains a sequence for AmpC beta-lactamase and may amplify for any assays designed to detect this antibiotic resistance target. For example, TaqMan™ Assay ID Ba04646128_s1 will amplify in any sample that contains the TaqMan™ Universal DNA Spike In Control.

TaqMan™ Universal DNA Spike In Control is supplied at a concentration of 200,000 copies/µL. The control is added during DNA isolation and is then carried through the remainder of the urinary tract microbiota profiling workflow. Coupled with the proprietary TaqMan™ Assay for the Xeno™ DNA control (Assay ID Ac00010014_a1), this verification layer helps ensure that PCR results are accurate, and it reduces the likelihood of false negatives.

For information about TaqMan™ Universal DNA Spike In Control, see *TaqMan™ Universal DNA Spike In Control Product Information Sheet* (Pub. No. MAN0017852).

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

TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*) (Cat. No. A39180), 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.

(Optional) Amplification control

The TaqMan™ Urinary Tract Microbiota Amplification Control (Cat. No. A39174) contains a linearized multi-target plasmid with target sequences for each available urinary tract microbiota profiling assay. The plasmid also contains target sequences for Xeno DNA and human RNase P RPPH1 genes, for a general control for the sample preparation process. The TaqMan™ Urinary Tract Microbiota Amplification Control can be included in urinary tract microbiota profiling experiments to verify assay performance and to help with troubleshooting.

For information about the amplification control, see *TaqMan™ Urinary Tract Microbiota Amplification Control Product Information Sheet* (Pub. No. MAN0017753).

TaqMan™ OpenArray™ Plate products and formats

TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate

The TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate (Cat. No. A39900) contains pre-plated, dried down TaqMan™ Assays for urinary tract microbiota profiling. For the complete lists of assays included with the TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate, see Table 1.

Contents and storage

Table 2 TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate (Cat. No. A39900)

Component	Amount	Storage
TaqMan™ Array Urinary Tract Microbiota Comprehensive Plate	1 plate	–25°C to –15°C

Custom TaqMan™ OpenArray™ Plate products and formats

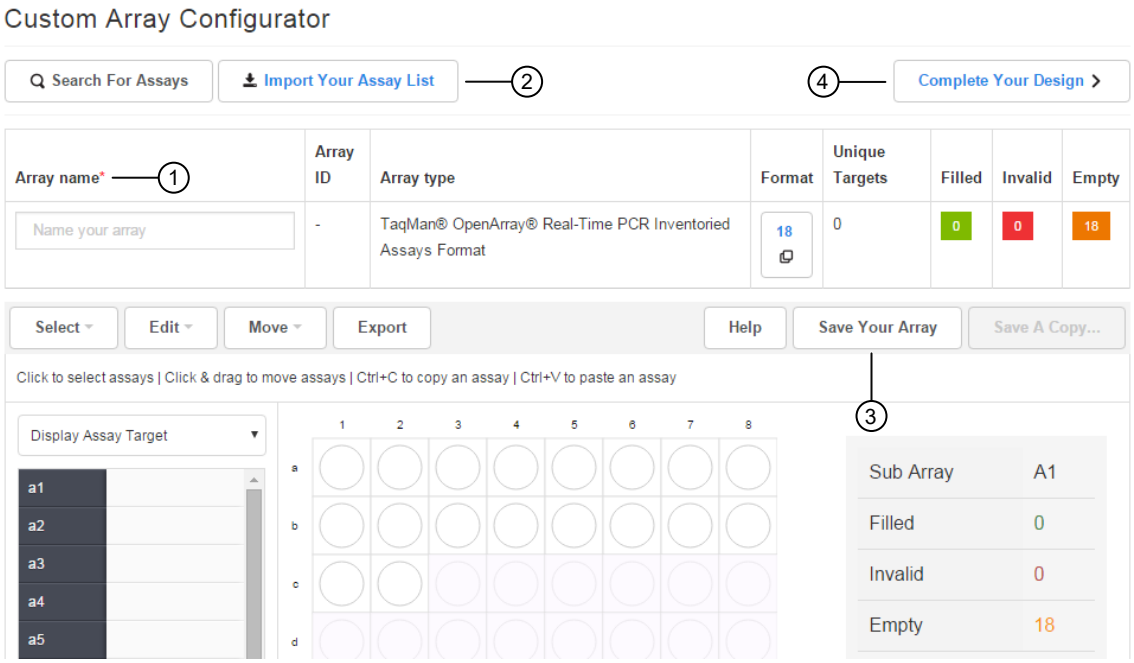
Custom TaqMan™ OpenArray™ Plates contain pre-plated, dried down TaqMan™ Assays for urinary tract microbiota profiling.

Array format	Number of assays	Maximum number of samples
18	18	48
56	56	48
112	112	24

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. In the table, click **Select** to configure a plate with the desired array format.
   The **Custom Array Configurator** screen is displayed.



① **Array Name** field
② **Import Your Assay List** button
③ **Save Your Array** button
④ **Complete Your Design** button

4. Enter the custom array name in the **Array name** text field.
5. 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**.
6. Follow the on-screen instructions to configure the assays on the plate.
7. *(Optional)* Click **Save Your Array** at any time to save the array configuration to your Thermo Fisher Scientific account.
8. When the plate is configured, click **Complete Your Design**, then follow the on-screen instructions to complete the order.

3 Isolate DNA from urine research samples using the MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit

Compatible sample collection and storage

Collect urine samples using BD™ Vacutainer™ urine collection cups and tubes.

- Compatible urine samples:
  - Unstabilized urine that is collected in sterile containers (BD™ Cat. No. 364975)
  - Urine that is collected and stored in Urine Analysis (UA) tubes (BD™ Cat. No. 364992)
  - Urine that is collected and stored in Culture and Sensitivity (C&S) tubes (BD™ Cat. No. 364951)
- *(Optional)* Store samples according to the instructions provided with the collection container, or use the following storage conditions:
  - Store at 4°C for up to one week.
  - Store at –80°C for long-term storage. We recommend storing samples in smaller volumes to prevent multiple freeze/thaw cycles.

Contents and storage

Reagents that are provided in the kit are sufficient for 100 reactions with standard volume input or 20 reactions with large volume input.

Table 3 MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit (Cat. No. A42356)

Component	Amount	Storage
Binding Solution	53 mL	15°C to 25°C
Wash Buffer	100 mL	
Elution Solution	10 mL	
Proteinase K	1 mL	
Total Nucleic Acid Binding Beads	2 mL	
Enzyme Mix	5 mL	–25°C to –15°C

Required materials not supplied for nucleic acid isolation

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.

Table 4 Required materials and equipment not included with the kit

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
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

Table 4 Required materials and equipment not included with the kit *(continued)*

Item	Source
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

Table 5 Additional materials and equipment required for processing urine samples

Item	Source
Centrifuge, capable of spinning deep-well plates at 2,250 × *g*	Fisher Scientific 75-412-452
Laboratory incubator with slatted shelves, capable of maintaining 65°C	MLS
(Optional) TaqMan™ Universal DNA Spike In Control (Xeno™ DNA control)	A39175
(Optional) TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*)	A39180

Procedural guidelines

IMPORTANT! Arrange plates in the incubator to allow adequate flow around the plate wells, to ensure that samples quickly reach and maintain the incubation temperature.

- 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.
- Use the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head and 96 deep-well heating block.
- When mixing samples by pipetting up and down, avoid creating bubbles.
- Cover the plate during the incubation and shaking steps to prevent spill-over and cross-contamination. We recommend a new MicroAmp™ Clear Adhesive Film for each step of the procedure.

- If you use a plate shaker other than the recommended shaker, confirm the following items:
  - The plate fits securely on your plate shaker.
  - The recommended speeds are compatible with your plate shaker. Ideal shaker speeds allow for thorough mixing without splashing.
- To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.
- Per-plate volumes for reagent mixes are sufficient for one plate plus overage. To calculate volumes for other sample numbers, refer to the per-well volume and add 5% overage.

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 sample layout

The sample plate layout provides sample tracking from the 96-well plate used for DNA isolation to the 96-well sample plate CSV file.

The sample plate layout is imported into the OpenArray™ Sample Tracker Software if OpenArray™ AccuFill™ Software v1.2 is used.

The sample plate layout is imported directly into OpenArray™ AccuFill™ Software v2.0.

Set up the sample plate layout using the CSV file described in the following table.

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

Tool	Source	Description
`96-well Sample Plate 1.csv`	On the computer on which the OpenArray™ Sample Tracker Software is installed: `<…>\Program Files (x86)\Applied Biosystems\OpenArray Sample Tracker\examples`, where <…> is the drive.	Contains a sample layout tab.
`96Well_Sample`	One the computer on which the OpenArray™ AccuFill™ Software v2.0 is installed: `<…>\Program Files\OpenArray AccuFill\resources\config`, where <…> is the drive.	

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

Concentrate the samples

1. Gently invert, shake, or swirl the sample contents to ensure thorough mixing of the sample.
2. Following the sample layout, transfer 1 mL of sample to the wells of a deep-well plate.
3. Seal the plate with a clear adhesive film, then centrifuge the plate at 2,250 × *g* for 15 minutes to concentrate the samples.
4. After centrifugation, carefully remove, then discard the supernatant.

   IMPORTANT! There may not be an obvious pellet. If a pellet is visible, be careful not to disturb the pellet.

   a. Set a P1000 pipette (or similar) to 900 µL.
   b. Angle the pipette so that the pipette tips sit at the bend from square to conical in the plate well.
   c. Carefully remove supernatant, then discard.
   d. Repeat substep 4b and substep 4c.
   e. Visually inspect the samples to ensure that all urine has been removed.

      A remaining volume of 30–100 µL is recommended. Some remaining supernatant is acceptable. It is more important to not disrupt the pellet.

      If too much supernatant remains, repeat substep 4b and substep 4c.

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 200 µL of PBS and 50 µL of Enzyme Mix to the concentrated samples of the KingFisher™ 96 Deep-Well Plate (Sample Plate).
3. (*Optional)* Add the TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*) to the appropriate wells.
   - 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 DNA Spike In Control (Xeno™ DNA 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 DNA Spike In Control (Xeno™ DNA 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.

4 Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v1.2

For required materials, see “Required materials for the OpenArray™ Plate workflow” on page 25.

For instructions for OpenArray™ AccuFill™ Software v2.0, see Chapter 5, “Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0”.

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

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

Workflow

Microbiota profiling experiments with OpenArray™ Plates

1. **Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software** (page 27)
2. **Set up the PCR reactions in an OpenArray™ 384-well Sample Plate** (page 28)
3. **Set up the OpenArray™ AccuFill™ Instrument and the OpenArray™ AccuFill™ Software** (page 29)
4. **Transfer reactions to the OpenArray™ Plate using the OpenArray™ AccuFill™ Instrument** (page 30)
5. **Seal the OpenArray™ Plate** (page 42)
6. **Run the OpenArray™ Plate on the QuantStudio™ 12K Flex Instrument** (page 45)
7. **Check the quality–control images** (page 46)

One-time procedures

Set up default folders and software preferences

This procedure simplifies the file locations used in the OpenArray™ AccuFill™ Software.

Set up the default file locations and preferences before using the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System for the first time.

1. Create the following four folders in a convenient location on the same computer drive as the OpenArray™ 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: `<…>\Program Files (x86)\Applied Biosystems\OpenArray Sample Tracker\examples`, where <…> is the drive.
   - 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 – 56.**
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 OpenArray™ AccuFill™ Software, select **Instrument ▸ Edit Preferences ▸ Require Sample Integration**, then select the folders indicated in this table:

OpenArray™ AccuFill™ Software 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 OpenArray™ 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 OpenArray™ AccuFill™ Software, transfer the loaded TPF files to the computer running the QuantStudio™ 12K Flex Software.

Download TPF files

Set up the optimized folder locations and software preferences before downloading TPF files. See “Set up default folders and software preferences” on page 22.

To download TPF files for custom OpenArray™ plates, you need the **Lot#** and the **Serial#** from the packaging of each OpenArray™ plate.

1. Go to **thermofisher.com/OA-platefiles**.
2. From the **Select Your Product** dropdown list, select **TaqMan™ OpenArray™ Custom Gene Expression/Genotyping Plates**.
3. Select the desired option for downloading either only the TPF files or both the TPF files and the AIF files.
4. Enter the **Lot#** and the **Serial#**, then click **Submit**.

   Note: The **Serial#** is case-sensitive.

5. Save the TPF files to the desktop **TPF Files** folder.

   Note: Do not create sub-folders in the **TPF Files** folder. The software cannot access sub-folders.

Required materials for the OpenArray™ Plate workflow

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

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

Item	Source
Instruments, software, and equipment	
OpenArray™ Sample Tracker Software (Not required for OpenArray™ AccuFill™ Software v2.0)	—[1]
QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0	A24945
QuantStudio™ 12K Flex Instrument with OpenArray™ block (QuantStudio™ 12K Flex OpenArray™ AccuFill™ System)	4471090
Centrifuge, capable of spinning sample plates at 1,200 × *g*	MLS
Plates and accessories	
OpenArray™ 384-well Sample Plates	4482221 (black) 4406947 (clear)
(Optional) 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
A sharp edge, blade, or scalpel (to cut the adhesive foil)	MLS
A fine-tip black marker for clear OpenArray™ 384-well Sample Plates A fine-tip silver or gold marker for black OpenArray™ 384-well Sample Plates	MLS
Reagents	
Genomic DNA	See page 14
(Optional) TaqMan™ Urinary Tract Microbiota Amplification Control	A39174
OpenArray™ Plates with TaqMan™ Assays	• A39900 • Custom ordered[3]
TaqMan™ OpenArray™ Real-Time PCR Master Mix	4462164

(continued)

Item	Source
100% molecular grade ethanol	MLS
Nuclease-free water	4387936

[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 "Configure and order CustomTaqMan™ OpenArray™ Plates" on page 13.

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 22 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.

      `<…>\Program Files (x86)\Applied Biosystems\OpenArray Sample Tracker\examples`, where <…> is the drive.

   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™ Urinary Tract Microbiota Amplification Control as a positive amplification control sample. For information about the amplification control, contact **GeneArtSupport@thermofisher.com**.

Component	OpenArray™ Plate Format	
	18	56
	Volume per well	Volume per well
TaqMan™ OpenArray™ Real-Time PCR Master Mix	2.5 µL	2.5 µL
DNA sample	2.5 µL	2.5 µL
Total reaction volume	**5.0 µL**	**5.0 µL**

Note: An overage is recommended when preparing the components.

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.

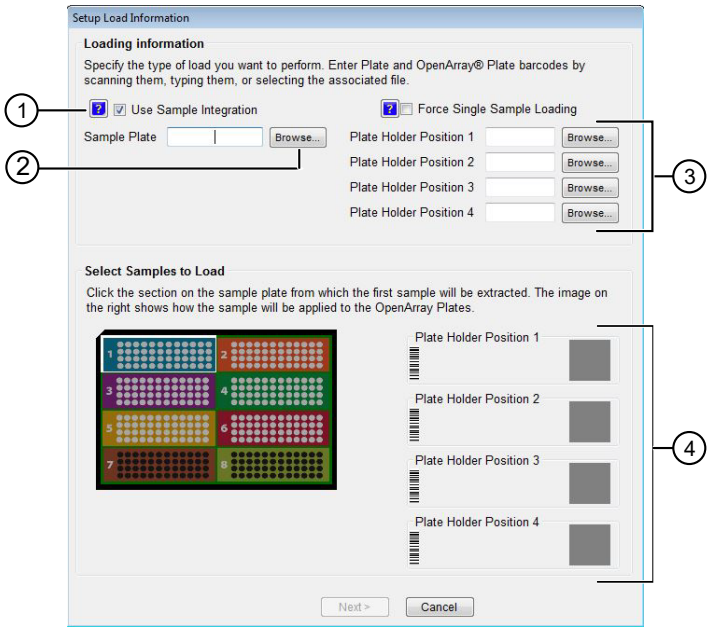
   Do not remove the foil from the scored area at this time.

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 OpenArray™ AccuFill™ Instrument and the OpenArray™ AccuFill™ 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 OpenArray™ 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 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 OpenArray™ 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 OpenArray™ 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 OpenArray™ 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 instrument.

4. In the OpenArray™ 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, then remove the loaded OpenArray™ Plate.

7. Proceed immediately to seal the OpenArray™ Plate.
   See “Seal the OpenArray™ Plate” on page 42.

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

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5 Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0

For required materials, see “Required materials for the OpenArray™ Plate workflow” on page 25.

For instructions for OpenArray™ AccuFill™ Software v1.2, see Chapter 4, “Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v1.2”.

This chapter describes the full run workflow. For other workflow options, see the following documentation.

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

Document	Pub. No.
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935
QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide	MAN0025669
OpenArray™ AccuFill™ Software v2.0 Quick Run Workflow Without Sample Information Quick Reference	MAN0025835
OpenArray™ AccuFill™ Software v2.0 Full Run Workflow Quick Reference	MAN0025836

Workflow

Microbiota profiling experiments with OpenArray™ Plates

- **Download TPF files** (page 34)
- **Set up the PCR reactions in an OpenArray™ 384-well Sample Plate (OpenArray™ AccuFill™ Software v2.0)**
- **Before you begin—full run workflow** (page 36)
- **Configure the experiment design for the full run workflow** (page 36)
- **Add or edit sample names** (page 37)
- **Verify the run setup and start the run** (page 37)
- **Remove the OpenArray™ Plate from the OpenArray™ AccuFill™ Instrument** (page 39)
- **Seal the OpenArray™ Plate** (page 42)
- **Run the OpenArray™ Plate on the QuantStudio™ 12K Flex Instrument** (page 45)
- **Check the quality–control images** (page 46)

Download TPF files

The TPF files are downloaded directly from **thermofisher.com/OA-platefiles** based on an order.

The computer with the OpenArray™ AccuFill™ Software v2.0 must be connected to the internet.

1. In the **TPF/SPF** screen, select the **Download** radio button.
2. Select the product.
   - **TaqMan OpenArray Custom**
   - **TaqMan OpenArray Inventoried**
3. Enter the following information.

Product	Information
TaqMan OpenArray Custom	a. Enter the ***Lot number*** or ***Batch number***. b. Enter one ***Serial number*** from the lot. **Note:** Only one serial number is required. The serial number is used to confirm the lot number or batch number. All of the files in the lot or batch are downloaded.
TaqMan OpenArray Inventoried	Enter the list of ***Serial numbers*** or ***Barcodes***. Separate more than one serial number or barcode with a comma or a line break. **Note:** The serial number or barcode entered corresponds to the file that is downloaded. Enter a serial number or barcode for each file to download.

Note: The fields that are displayed depend on the product selected in step 2.

4. *(Custom Gene Expression plates only)* Select one of the following options:
   - **With microbial target names**
   - **Without microbial target names**

Note: The microbial target name selection is not displayed if inventoried products are selected.

5. Click **Download**.

The location of the files is displayed at the top of the screen. The location of the downloaded files is set in the **Preferences** menu, in the **OpenArray plate file folder** field. See *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669) for more information about setting the preferences.

The files are in a compressed ZIP folder.

Click **Open folder** to access the files or click ✕ **(Close)** to close the message.

Extract the files from the compressed ZIP folder.

Set up the PCR reactions in an OpenArray™ 384-well Sample Plate (OpenArray™ AccuFill™ Software v2.0)

IMPORTANT! The 4 × 12 area(s) of the OpenArray™ 384-well Sample Plate being filled must match the area(s) designated in the OpenArray™ AccuFill™ 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 vortex the bottle.

3. Following the designated sample plate layout, add master mix, then DNA samples, to the wells of an OpenArray™ 384-well Sample Plate.

   (Optional) Use the TaqMan™ Urinary Tract Microbiota Amplification Control as a positive amplification control sample. For information about the amplification control, contact GeneArtSupport@thermofisher.com.

Component	OpenArray™ Plate Format	
	18	56
	Volume per well	Volume per well
TaqMan™ OpenArray™ Real-Time PCR Master Mix	2.5 µL	2.5 µL
DNA sample	2.5 µL	2.5 µL
Total reaction volume	**5.0 µL**	**5.0 µL**

Note: An overage is recommended when preparing the components.

4. Thoroughly mix each PCR reaction by pipetting up and down or by using the "mix" function on a multi-channel pipette. Alternatively, vortex the OpenArray™ 384-well Sample Plate for 10–15 seconds after sealing with aluminum foil in step 5.

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, it is possible to correct this in the OpenArray™ AccuFill™ Software using the plate rotation feature. For more information, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

Before you begin—full run workflow

- Prepare samples in an OpenArray™ 384-well plate (see "Set up the PCR reactions in an OpenArray™ 384-well Sample Plate (OpenArray™ AccuFill™ Software v2.0)" on page 35).
- Place the sample plate in the sample plate holder on the AccuFill™ instrument deck, with the notch to the left.

  Do not stack sample plates.
- Load the tip boxes, then remove the tip box covers.

  Do not stack the tip boxes.
- Place the OpenArray™ Plates in the plate holders.
- Clear the instrument deck, empty and replace the waste bin, then close the instrument door.
- Allow the instrument to perform a self-test if the run is being started after the software is launched.
- Prepare the QuantStudio™ 12K Flex OpenArray™ Accessories Kit materials prior to uncovering tip boxes and removing OpenArray™ plates from packaging.

  These materials are used to seal the OpenArray™ Plates.

  IMPORTANT! OpenArray™ Plates must be sealed immediately after loading.

Configure the experiment design for the full run workflow

A TPF file *is* required for this workflow.

Navigate to the **Full Run** screen.

1. In the **Configure design** pane, in the **Experiment type** section, select **Gene expression**.
2. In the **Plate format** section, select a format.

   The values in the **Plate format** section depend on the experiment type that was selected in step 1.
3. If the **Pipettor** section is displayed, select a type of pipette.
   - **Fixed**
   - **Adjustable**
4. In the **Add your OpenArray Plate serial numbers** section, click **Choose File**, navigate to the location of the TPF file, then select the file.

   Repeat for each TPF file.

5. In the **Add your sample plates - optional** section, click **Choose File**, navigate to the location of the CSV file, then select the file.
   The format of the sample plate file is validated. For information about the required format, see the *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).
   The name of the file is displayed in the **Select file** field.
6. Repeat step 5 for each CSV file.
7. Click **Next**.

The **Map plates** pane is displayed.

Proceed to "Add or edit sample names" on page 37.

Add or edit sample names

If needed, navigate to the **Map plates** pane in the **Full Run** screen.

If a sample plate file was imported, the sample names are displayed. The sample plate layout defined in the sample plate file can be edited.

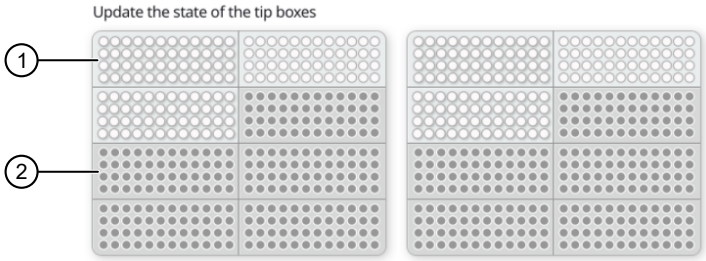
If the sample plate file was not imported, the samples must be added manually.

1. Add or edit the sample name.
2. Click **Next**.

Proceed to "Verify the run setup and start the run" on page 37.

Verify the run setup and start the run

1. Click each tip box section so that the status on the **Verify and start run** pane matches the physical tip box in the instrument.
   We recommend starting the run with full tip boxes. The instrument does not start the run if there are not enough tips on the deck.



① Section of the tip box that is full.
② Section of the tip box that is empty.

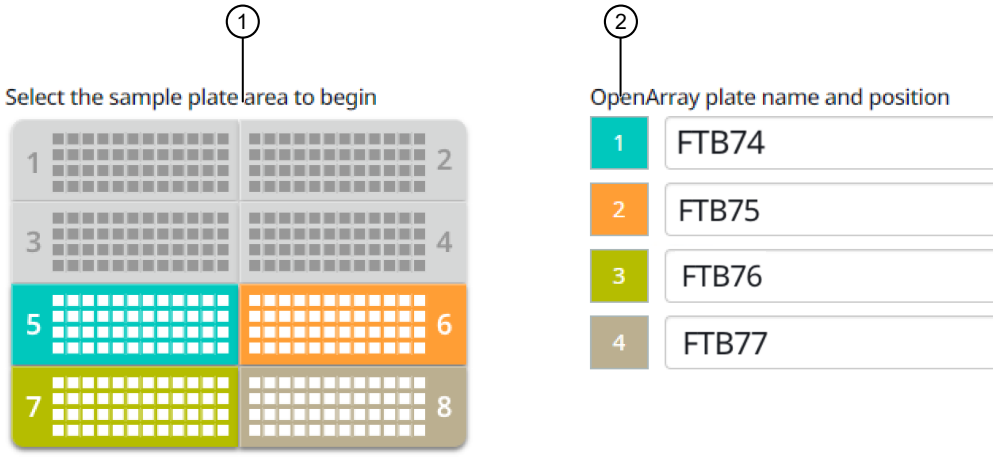
2. *(Optional)* Click **Auto-fill tip boxes**.
   The status of all sections of the tips boxes is set to full.

3. Select the first section of the sample plate to be used to fill the OpenArray™ Plate.

   Select the first section of the sample plate if multiple plates are filled during a run. The software selects the total number of sections that correspond with the total number of plates.

   In the following example, section 5 was selected. The group of sections 5, 6, 7, and 8 is highlighted by the software because four plates are being filled.

   The position box displays the color that corresponds to the section of the sample plate.



① Sample plate section (section 5, 6, 7, and 8 are highlighted).
② Corresponding plates.

4. Remove the foil from the appropriate sections of the sample plate, then click the checkbox to confirm.

   Remove the foil only from the sections of the sample plate that are used to load a single OpenArray™ Plate.

   Note: Do not remove the foil from all the sections of the sample plate at once.

5. Close the instrument door.

6. Click **Start Run**.

   The run does not begin under any of these conditions.

   - The waste bin is not in position.
   - The sample plate is not in position.
   - The plates are not in position.
   - There are more plates on the instrument deck than are defined in the experiment setup.

The **Deck** screen is displayed.

For a description of the run progress, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

IMPORTANT! Each OpenArray™ Plate must be prepared for PCR immediately after it is filled (see “Remove the OpenArray™ Plate from the OpenArray™ AccuFill™ Instrument” on page 39).

Remove the OpenArray™ Plate from the OpenArray™ AccuFill™ Instrument

After an OpenArray™ Plate is filled, the **Remove plate and foil** dialog box is displayed (see Figure 1).

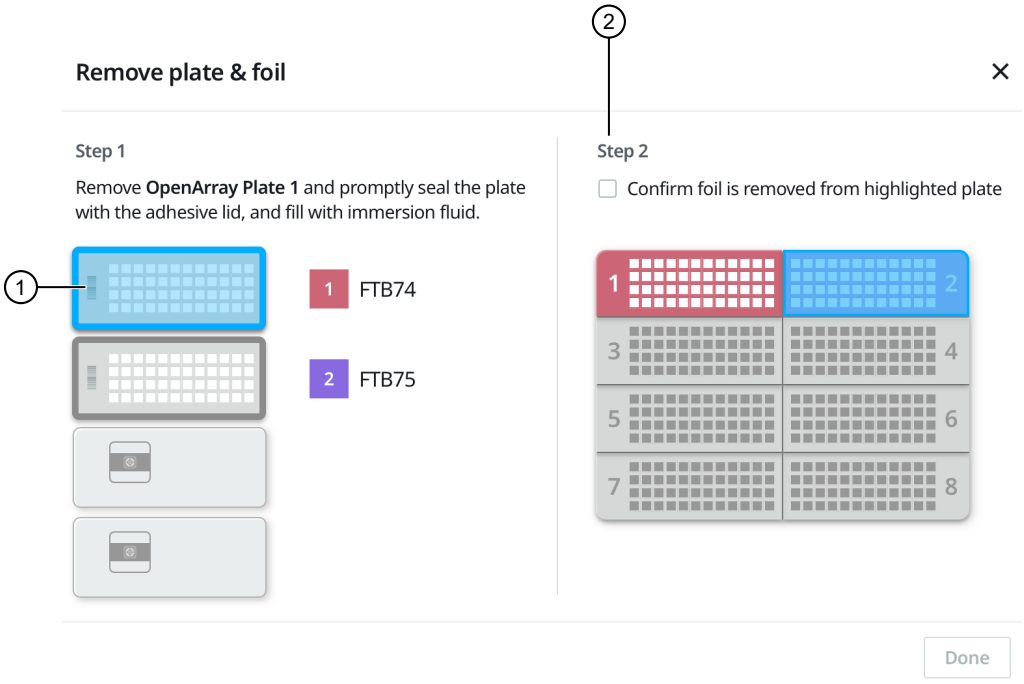


Figure 1 Remove plate and foil dialog box.

① OpenArray™ Plate to remove from the instrument.
② **Confirm foil is removed from highlighted plate section** checkbox.

Remove each OpenArray™ Plate *immediately* after it has been filled, even if the run was set up to fill multiple plates.

After the last OpenArray™ Plate in the run is filled, the **Remove plate** dialog box is displayed (see Figure 2).

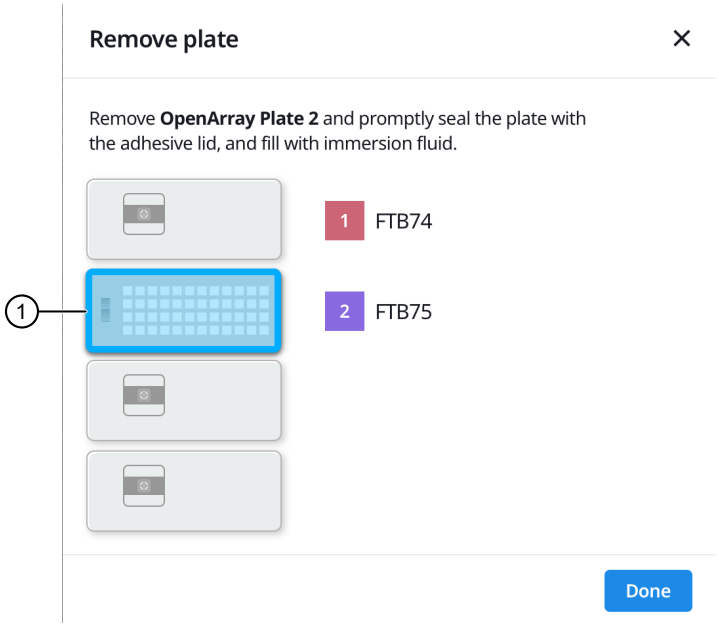


Figure 2 Remove plate dialog box

① OpenArray™ Plate to remove from the instrument

1. Open the instrument door and remove the OpenArray™ Plate that is indicated by the blue box in the dialog box.

   IMPORTANT! Remove the OpenArray™ Plate immediately, to avoid evaporation within the plate.

   One of the following dialog boxes is displayed:

   - The **Remove plate and foil** dialog box.
   - The **Remove plate** dialog box (after the last OpenArray™ Plate is filled).

2. Seal the case and fill the OpenArray™ Plate with immersion fluid.

   See "Seal the OpenArray™ Plate" on page 42.

3. *(For **Remove plate and foil** dialog box only)* Remove the foil seal from the next section of the sample plate, then select the checkbox to confirm that the foil is removed from the section of the plate that is highlighted.

   Note: Remove the foil only from the next section of the sample plate. Do not remove the foil from all sections of the sample plate.

4. Close the instrument door.

5. Click **Done**.

   The run does not proceed under any of the following conditions:

   - The waste bin is not in position
   - The sample plate is not in position
   - The plates are not in position
   - There are more plates on the instrument deck than are defined in the experiment setup

   The instrument proceeds to load the next OpenArray™ Plate.

6. Repeat step 1 to step 5 for each OpenArray™ Plate to be loaded.

After all of the plates have been loaded, the **Deck** screen displays **Run completed successfully. Empty the waste bin before performing another run.**

A loaded TPF is generated for each OpenArray™ Plate. The loaded TPF file corresponds to the original TPF file that was imported for the run. The files are exported to the folder that was designed in the **Preferences**.

Note: Some workflows might not generate a loaded TPF file. For more information about the workflows available for the OpenArray™ AccuFill™ Software v2.0, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

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6 Seal and run the OpenArray™ Plates

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 ① and the red adhesive-protective strip ② from around the edge of the lid.

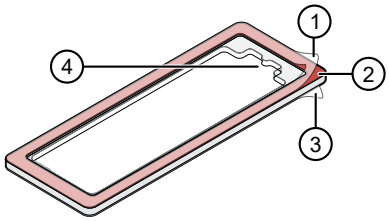
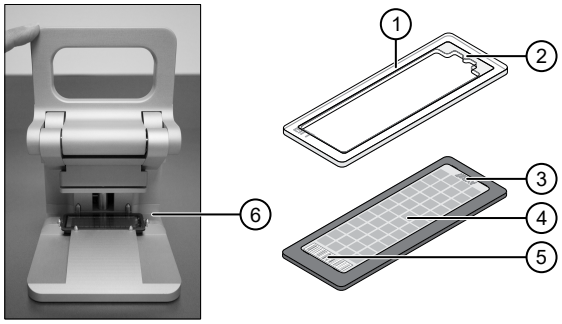


Figure 3 OpenArray™ Lid

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. Place the lid in the Plate Press using the alignment pins of the Plate Press for orientation.

IMPORTANT! The notched end of the case lid must be oriented towards the furthest back right-side of the Plate Press.



① OpenArray™ case lid
② Notched end of lid
③ Serial number of plate
④ OpenArray™ Plate
⑤ Barcode of plate
⑥ Alignment pins

4. Seat the lid on the OpenArray™ Plate with the lid adhesive against the plate.

5. Engage the press mechanism until the green flashing light changes to a steady green light (after 20 seconds).

   The status light turns solid green, indicating that the case is sealed.

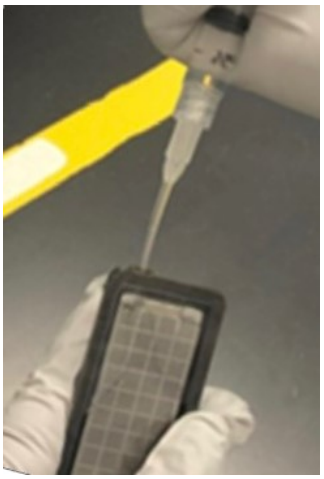
Note: Do not apply additional pressure onto the Plate Press during its actuation.

6. Disengage the press and carefully remove the OpenArray™ Case.

7. Prepare the immersion fluid. Remove the cap, insert the accompanying syringe tip, and prime the syringe by ejecting a small amount of immersion fluid onto a paper towel to ensure no air gap remains in the newly attached pipette tip.

IMPORTANT! If the syringe is not primed, the direct burst of air and fluid can negatively affect the assay(s) at the end of the array.

8. While holding the case upright by its edges at a 15–30 degree angle so that the port is at the highest point of the array, insert the prepared syringe tip into the port in the case.

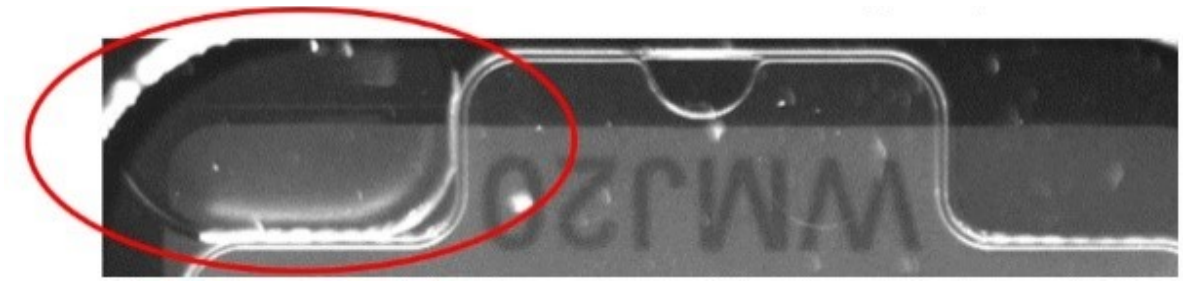


The syringe tip must be in front of the array when filling the case with immersion fluid.

9. Slowly inject the OpenArray™ Immersion Fluid until the case is filled, which should take about 10 seconds to fill. Minimize the creation of additional air bubbles when you dispense the fluid. Leave a small air bubble as shown below.

IMPORTANT! If injected too quickly, the fluid can flush out the samples that are suspended in the through-holes.

Overfilling the array and/or not leaving a small bubble may cause a leak during the PCR run.



10. 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.

    Note: Ensure that you are screwing the plug in at the same angle the case base is at. If it is off, it can cause the plug to break off prematurely.

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

    If the plug handle breaks off prematurely, use a Phillips #0 screwdriver to complete this step. Do not overtighten. If plastic or adhesive remains attached to the screw due to premature breakout of the plug handle, remove it with forceps prior to loading it into the instrument.

11. If needed, clean the case with the lint-free cloth included with the OpenArray™ Plate or 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.
   - Ensure that the OpenArray™ Plate adapter A1 position is aligned with the instrument arm adapter A1 position.
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 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**.

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.

7 Export and review urinary tract microbiota profiling data

Export data

1. Open an EDS file in the QuantStudio™ 12K Flex Software.
2. In the **Experiment Menu** pane, in the **Analysis** tab, click **Analyze**.
3. In the **Experiment Menu** pane, click  **Export**.
4. Click **Load Export Set** (bottom of the screen), select **GE_export_setting**, then click **OK**.
5. Select **.xlsx** from the **File Type** dropdown list (top-right of the screen).
6. *(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.
7. 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.

Prepare exported data for analysis

1. Open the exported XLSX data file.
2. Ensure that the barcode, run conditions, and all selected data columns were exported correctly.
3. Scroll down to the data rows, select the headers and data, then copy-paste into a new worksheet.
4. Rename the new worksheet **Data Table_*Run File Name***.

5. *(Optional)* To combine data from multiple OpenArray™ Plates, perform the following steps:
   a. Insert a **Barcode** column in the **Data Table** worksheet to track OpenArray™ barcodes.
   b. Copy-paste the barcode numbers to the appropriate cells in the new **Barcode** column.
6. Find-replace all "**Undetermined**" values with an empty cell (no value) in the **C_{rt}** column. This step ensures an exact count of C_{rt} values.
7. Delete rows that do not contain run data.

Review results

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

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

1. Review the exported data for through-hole results that may require special attention.
2. Consider filtering out from analysis through-holes with the following values:

Parameter to examine	Consider filtering out through-holes if…
C_{rt}	**C_{rt}** ≥ 31
C_q Confidence	**C_q Conf** < 0.8 • RNase P (Hs04930436_g1) — acceptable range is 0.7 – 1.0
Amp Score	Amp Score < 1.2

Note: Through-holes with unexpected C_{rt} values can also be identified by reviewing the Amplification Plot (see page 50).

3. Review through-holes with $C_{rt} > 28$ and ensure that the C_{rt} values are reproducible in all technical replicates.

Note: $C_{rt} = 28$ is approximately equal to 1 copy of the target sequence in a reaction.

4. Take note of technical replicates with mean $C_{rt} \leq 25$ and a high 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.

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}
	—	Average of Amp Score
	—	Average of C_q Conf

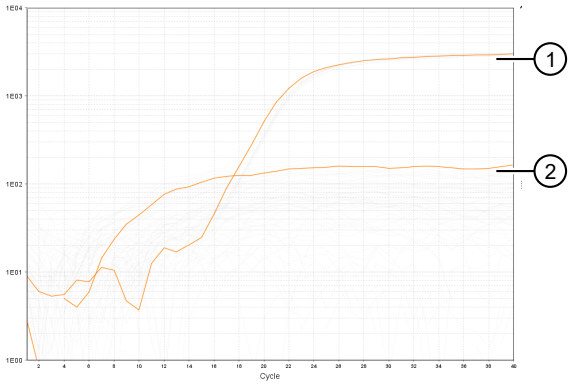
[1] To see individual sample results, select the sample from the dropdown list next to the **Sample Name** header.

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

A

Troubleshooting

Troubleshoot unexpected C_{rt} values

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, AmpScore, and C_q Confidence values.
		Consider filtering C_{rt} values from analysis.
		Compare replicates, if available.
		Repeat the experiment.
	Unexpectedly high C_{rt} values (< 28) — Sporadic amplification	Review amplification curves, AmpScore, and C_q Confidence values.
		Consider filtering C_{rt} values from analysis if < 50% of replicates amplified at similar levels.
		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.

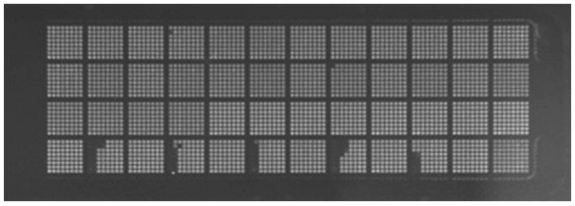
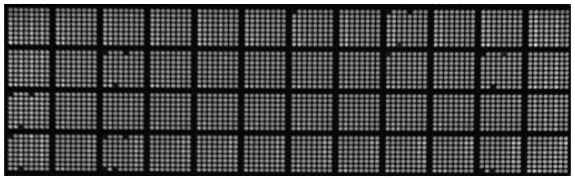
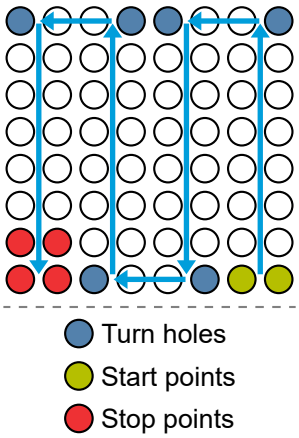
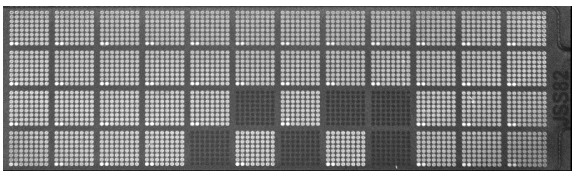
2. Use ImageJ to view the images of interest.

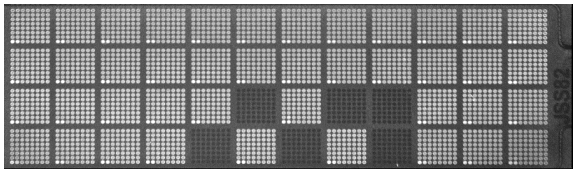
To...	View image...	Image description
Confirm the identity of images within a folder	BARCODE IMAGE.tiff	Reflected light image of the entire OpenArray™ Plate.
Evaluate the loading quality	PRE-READ_CHANNEL_4.tiff POST-READ_CHANNEL_4.tiff	Pre- and post-ROX™ dye images.
Check for existing contamination on 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.

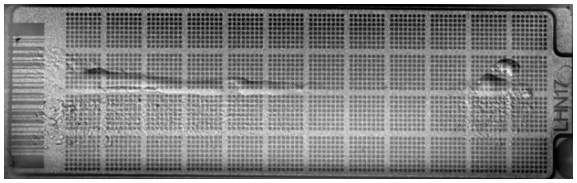
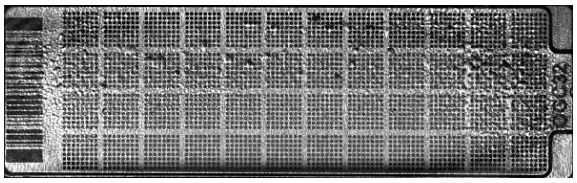
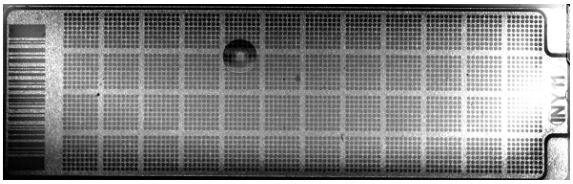
3. *(Optional)* Adjust the images for brightness and/or contrast to make image features visible.
   a. Open the image in ImageJ.
   b. Select **Image** ▸ **Adjust Brightness/Contrast** (or press **Ctrl+Shift+C**).
   c. Click **Auto** or adjust the sliders until the features of interest in the image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

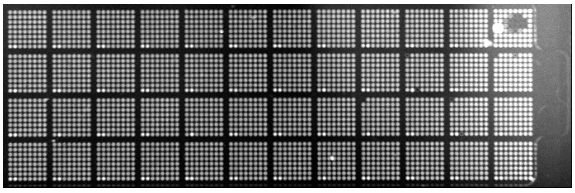
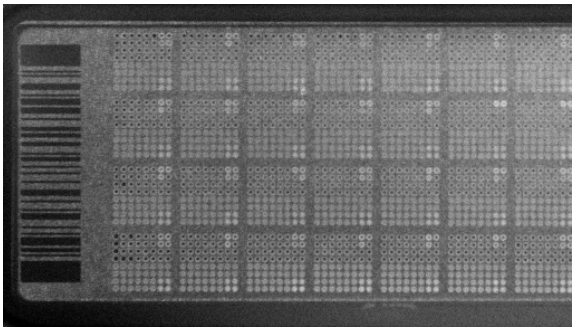
OpenArray™ AccuFill™ Instrument plate loading errors

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 	The OpenArray™ AccuFill™ Instrument is aligned too far to the left or to the right. Systematic loading problems can occur with the OpenArray™ 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 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 the OpenArray™ AccuFill™ Instrument may need cleaning.	Contact your local field service engineer.

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

OpenArray™ Plate assembly and handling errors

Observation	Possible cause	Recommended action
Case leaks and bubbles inside the case    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. 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	Plate press was not engaged for at least 20 seconds.	Fully engage the plate press for at least 20 seconds.
	Damaged lid adhesive.	Remove the liner and visually inspect the lid adhesives for defects. Ensure that adhesive is not damaged or warped.
	Damaged fill port screw gasket.	Visually inspect the screw to ensure that the orange gasket is present and not damaged.
	Damaged fill port screw assembly. Breaks off too easily.	The screw may be mis-threaded: unscrew it and use a new screw assembly.
	Oily lid or case from immersion fluid overflow.	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).
	Immersion fluid was exposed to air for too long.	Do not remove the immersion fluid syringe cap or draw air bubbles into the syringe until you are ready to load.

Observation	Possible cause	Recommended action
windows and obscured the view of the through-holes. 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 51.	Too large of a bubble inside the OpenArray™ Case after sealing.	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.
	Damaged plate press, leading to uneven pressure.	Contact your field service engineer if you suspect that your plate press may be damaged.
Sample blow-out during the addition of immersion fluid 	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.	Dispense a small amount of immersion fluid onto a paper towel before use to ensure smooth operation of the syringe.
Evaporation of reaction mixture in through-holes 	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.	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.

B

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.

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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

C Documentation and support

Related documentation

Document	Publication Number
DNA isolation for Urinary Tract Microbiota Profiling Experiments Quick Reference	MAN0017751
Urinary Tract Microbiota Profiling Experiments using OpenArray™ and AccuFill™ Software v1.2 Quick Reference	MAN0017752
Urinary Tract Microbiota Profiling Experiments using OpenArray™ and AccuFill™ Software v2.0 Quick Reference	MAN0026012
TaqMan™ Urinary Tract Microbiota Amplification Control Product Information Sheet	MAN0017753
TaqMan™ Universal Extraction Control Organism (B. atrophaeus) Product Information Sheet	MAN0018535
TaqMan™ Universal DNA Spike In Control Product Information Sheet	MAN0017852
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935
QuantStudio™ 12K Flex Real–Time PCR System Maintenance and Administration Guide	4470689
QuantStudio™ 12K Flex Real–Time PCR System v1.5 or later Maintenance and Administration Guide	MAN0018832
Thermo Scientific™ KingFisher™ Flex User Manual	MAN0019870
OpenArray™ Sample Tracker Software Quick Reference, for OpenArray™ AccuFill™ Software v1.2	4460657
OpenArray™ AccuFill™ System User Guide, for OpenArray™ AccuFill™ Software v1.2	4456986
QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide, for OpenArray™ AccuFill™ Software v2.0	MAN0025669

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**.

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