appliedbiosystems

Vaginal Microbiota Profiling Experiments

APPLICATION GUIDE

for use with:

TaqMan™ OpenArray™ Plates

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

Publication Number MAN0015669

Revision E

For Research Use Only. Not for use in diagnostic procedures.

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

Revision history: MAN0015669 E (English)

Revision	Date	Description
E	18 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 32). • Sealing instructions were updated ("Seal the OpenArray™ Plate" on page 39). • Minor verbiage updates throughout document.
D.0	17 January 2023	Changes to remove the A32039: TaqMan® Vaginal Microbiota Extraction Control, as this product is being discontinued.
C.0	13 January 2022	• The instructions to configure and order Custom TaqMan™ OpenArray™ Plates were updated. • OpenArray™ 384-well Sample Plates (clear) were added as an option. • The Biomek™ Seal and Sample Foil Lids were changed to an optional material. • 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 MagMAX™ DNA Multi-Sample Ultra Kit is still the recommended kit for isolating DNA from samples collected with the Hologic™ Aptima™ Vaginal Swab Transport Media (STM). • The BD SurePath™ test was removed as a compatible sample collection system. • B-PER™ Bacterial Protein Extraction Reagent, Lysozyme Solution, and Zymolyase were removed from the list of required materials for the main workflow. They are not required for the DNA isolation procedure with the MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit. These reagents are required for isolating DNA from samples collected with the Hologic™ Aptima™ Vaginal Swab Transport Media (STM). • The procedural guidelines were updated to recommend a new MicroAmp™ Clear Adhesive Film for each step of the procedure. • The location of the sample plate layout for OpenArray™ Sample Tracker Software was corrected (applies to OpenArray™ AccuFill™ Software v1.2 only). • The time to shake a plate when digesting the samples with Proteinase K during the DNA isolation was changed from 2 minutes to 3 minutes in the procedure for samples collected with the Hologic™ Aptima™ Vaginal Swab Transport Media (STM). • The step to centrifuge the plate if condensation is present before adding DNA Binding Beads and RNase A was updated to be optional in the procedure for samples collected with the Hologic™ Aptima™ Vaginal Swab Transport Media (STM). • A step was added to mix the PCR reaction when setting up the 384-well sample plate for OpenArray™ AccuFill™ Software v1.2. • The centrifuge speed was updated when preparing PCR reactions in an OpenArray™ 384-well Sample Plate and when troubleshooting empty through-holes. • A step was added to score the foil when setting up the 384-well sample plate for OpenArray™ AccuFill™ Software v1.2. • The recommended action of comparing replicates was added to troubleshooting unexpected C_{rt} values. • The instructions to seal the OpenArray™ Plates were updated. The protective film on the outside of the lid is removed after sealing the OpenArray™ Plates, but before performing real-time PCR. • Instructions were added for the OpenArray™ AccuFill™ Software v2.0. • The instructions to recover from sample plate layout errors were removed. • The instructions to export the data were corrected to include a step to analyze the data before they are exported.
B.0	9 November 2016	• Add information about the optional extraction and amplification controls. • Remove Remel™ M4™ MicroTest™ from the list of compatible sample collection systems. • Update optional pipetting instructions during digestion with Preliminary Digestion Mix. • Clarify the guidelines for reviewing the results. • General streamlining of content for ease of use and readability.

Revision	Date	Description
A.0	28 June 2016	New Document

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

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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 vaginal microbiota profiling. The workflow uses:

- OpenArray™ Plates with TaqMan™ Assays for vaginal microbiota profiling
- The QuantStudio™ 12K Flex Instrument with OpenArray™ block (QuantStudio™ 12K Flex OpenArray™ AccuFill™ System)

Vaginal microbiota profiling

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

We offer a collection of qualified TaqMan™ Assays (see “TaqMan™ Assays for vaginal microbiota profiling” on page 12) that have been optimized for detection of vaginal microbes. The TaqMan™ Assays designs and their target sequences have undergone rigorous bioinformatics selection and analysis to allow maximum strain coverage while minimizing the potential for off-target cross-reactivity. Qualified TaqMan™ Assays for vaginal microbiota profiling demonstrate accurate, reproducible performance in multiple rounds of testing for sensitivity and specificity. The assays perform well with DNA isolated from vaginal samples using the MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit.

Additional TaqMan™ Assays for microbial targets are available from our predesigned assay collection or can be ordered through the Custom TaqMan™ Assay Design Tool.

Materials required but not supplied

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

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

Required materials not supplied

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

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

Table 1 Required materials and equipment not included with the kit

Item	Source
One of the following instruments	
(Recommended) KingFisher™ Flex Magnetic Particle Processor	5400630
MagMAX™ Express-96 Magnetic Particle Processor	—[1]
Equipment	
Plate shaker, capable of shaking plates at a minimum of 900 rpm	88880023
Analog Vortex Mixer	Fisher Scientific 02-215-365
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
(Optional) Magnetic Stand-96	AM10027
Plates and combs	
Deep Well Plates, one of the following:	
KingFisher™ Flex Microtiter Deep-Well 96 plates, sterile	95040460
MagMAX™ Express-96 Deep Well Plates	4388476
Standard Well Plates, one of the following:	
KingFisher™ 96 KF microplates	97002540
MagMAX™ Express-96 Standard Plates	4388475
Tip Combs, one of the following:	
KingFisher™ 96 tip comb for DW magnets	97002534
MagMAX™ Express-96 Deep Well Tip Combs	4388487
Other consumables	
MicroAmp™ Clear Adhesive Film	4306311
RNase-free Microfuge Tubes (2.0 mL)	AM12425
Conical tubes (15 mL)	AM12500
Conical tubes (50 mL)	AM12502

Item	Source
Aerosol-resistant pipette tips	MLS
Reagent reservoirs	MLS
(Optional) Paraffin film	MLS
Reagents	
Ethanol, 200 proof (absolute)	MLS
Isopropanol, 100% (molecular grade or higher)	MLS

[1] Not available for sale.

Table 2 Additional materials and equipment required for processing vaginal 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

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)	4471021
Centrifuge, capable of spinning sample plates at 1,200 × *g*	MLS
Plates and accessories	
OpenArray™ 384-well Sample Plates, Black *or* OpenArray™ 384-well Sample Plates	4482221 4406947
(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
Reagents	
Genomic DNA	See page 16
(*Optional*) TaqMan™ Vaginal Microbiota Amplification Control	A32040
OpenArray™ Plates with TaqMan™ Assays	Custom ordered[3]
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 is shipped with an accessories kit.

[3] See “Configure and order CustomTaqMan™ OpenArray™ Plates” on page 14.

Workflow: TaqMan™ vaginal microbiota profiling experiments

Collect vaginal sample using a compatible system or media (see "Compatible sample collection systems or media" on page 17)

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

▼

Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v1.2 (page 22) OR Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0 (page 30)

▼

Seal and run the OpenArray™ Plates (page 39)

▼

Export and review vaginal microbiota profiling data (page 44)

2 Background and tools for assay content selection

TaqMan™ Assays

TaqMan™ Assays for vaginal 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, visit **thermofisher.com/qpcreducation**.

TaqMan™ Assays for vaginal microbiota profiling

OpenArray™ plates can be configured with the following TaqMan™ Assays.

For more information about available TaqMan™ Assays for vaginal microbiota profiling, visit **thermofisher.com/vm**.

Table 3 TaqMan™ Assays for vaginal microbiota profiling

Assay ID	Classification	Organism
Ba04646222_s1	Bacteria	*Atopobium vaginae*
Ba04646225_s1	Bacteria	*Bacteroides fragilis*
Ba04646229_s1	Bacteria	*BVAB2*
Ba04646249_s1	Bacteria	*Chlamydia trachomatis*
Ba04646247_s1	Bacteria	*Enterococcus faecalis*
Ba04646242_s1	Bacteria	*Escherichia coli*
Ba04646236_s1	Bacteria	*Gardnerella vaginalis*

Table 3 TaqMan Assays for vaginal microbiota profiling *(continued)*

Assay ID	Classification	Organism
Ba04646228_s1	Bacteria	*Haemophilus ducreyi*
Ba04646245_s1	Bacteria	*Lactobacillus crispatus*
Ba04646234_s1	Bacteria	*Lactobacillus gasseri*
Ba04646257_s1	Bacteria	*Lactobacillus iners*
Ba04646258_s1	Bacteria	*Lactobacillus jensenii*
Ba04646230_s1	Bacteria	*Megasphera 1*
Ba04646231_s1	Bacteria	*Megasphera 2*
Ba04646235_s1	Bacteria	*Mobiluncus curtisii*
Ba04646246_s1	Bacteria	*Mobiluncus mulieris*
Ba04646251_s1	Bacteria	*Mycoplasma genitalium*
Ba04646255_s1	Bacteria	*Mycoplasma hominis*
Ba04646252_s1	Bacteria	*Neisseria gonorrhoeae*
Ba04646278_s1	Bacteria	*Prevotella bivia*
Ba04646259_s1	Bacteria	*Staphylococcus aureus*
Ba04646276_s1	Bacteria	*Streptococcus agalactiae* (group B)
Ba04646237_s1	Bacteria	*Treponema pallidum* (Syphilis)
Ba04646254_s1	Bacteria	*Ureaplasma urealyticum*
Fn04646233_s1	Fungi	*Candida albicans*
Fn04646244_s1	Fungi	*Candida dubliniensis*
Fn04646240_s1	Fungi	*Candida glabrata*
Fn04646250_s1	Fungi	*Candida krusei*
Fn04646241_s1	Fungi	*Candida lusitaniae*
Fn04646221_s1	Fungi	*Candida parapsilosis*
Fn04646220_s1	Fungi	*Candida tropicalis*
Pr04646256_s1	Protozoa	*Trichomonas vaginalis*
Vi04230116_s1	Virus	HSV1
Vi04646232_s1	Virus	HSV2

Optional controls

(*Optional*) Amplification control

The TaqMan™ Vaginal Microbiota Amplification Control (Cat. No. A32040) contains a linearized multi-target plasmid with target sequences for each available vaginal microbiota profiling assay. The plasmid also contains target sequences for the prokaryotic 16S rRNA and human RNase P RPPH1 genes, for general detection of bacterial and human DNA, respectively. The TaqMan™ Vaginal Microbiota Amplification Control can be included in vaginal microbiota profiling experiments to verify assay performance and to help with troubleshooting.

For information about the amplification control, see the *TaqMan™ Vaginal Microbiota Amplification Control Product Information Sheet* (Pub. No. MAN0016007).

(*Optional*) Reference and control assays

The following TaqMan™ Assays are available as optional reference or control assays for vaginal microbiota profiling experiments. These assays can be used in relative quantification applications and to assess the adequacy of the human sample.

Assay ID	Target	Application
Ba04930791_s1	Prokaryotic 16S rRNA	• Relative quantification/normalization to bacterial DNA
Hs04930436_g1	Human RNase P RPPH1 gene	• Relative quantification/normalization to human DNA • Assessment of sample adequacy

OpenArray™ Plate products and formats

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

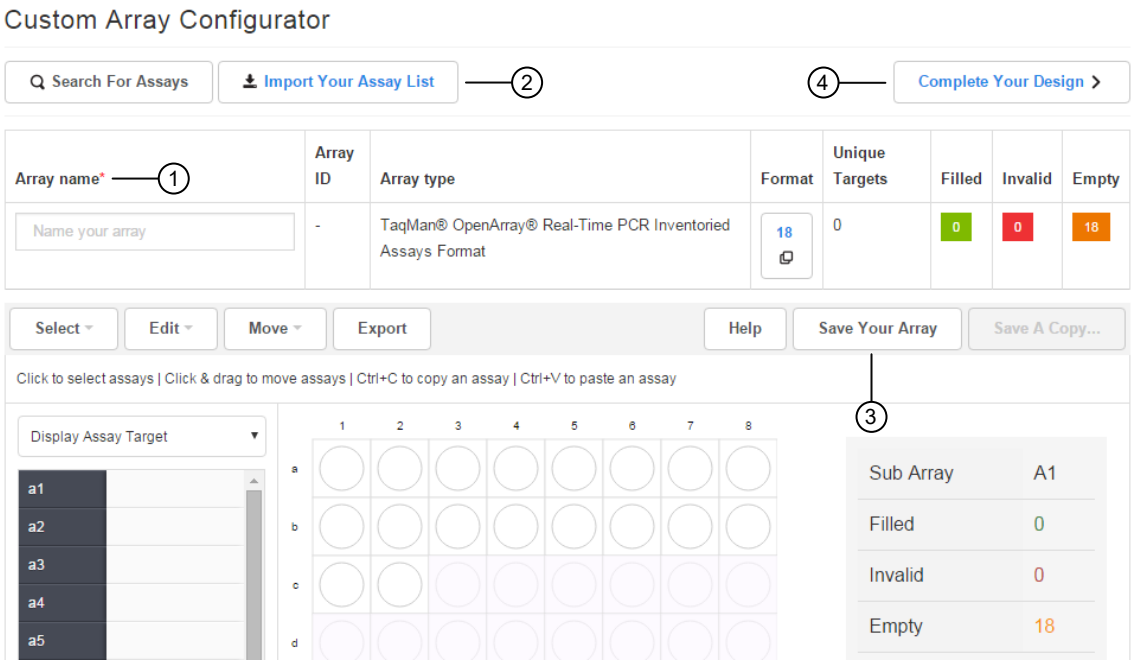
Array format	Number of assays	Maximum number of samples
18	18	48
56	56	48
112	112	24

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 using the MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit

IMPORTANT! Samples collected using the Hologic™ Aptima™ Vaginal Swab Transport Media (STM) require a different DNA isolation procedure (see Appendix B, “Isolate DNA using the MagMAX™ DNA Multi-Sample Ultra Kit: Hologic™ Aptima™ media”).

Compatible sample collection systems or media

The following sample collection systems and media are compatible with the procedures described in this guide.

See the collection system or media documentation provided by the manufacturer for information on sample collection and storage.

Sample collection system / media	Source
ThinPrep™ Pap test	Hologic™
eSwab™[1,2]	Copan™
Aptima™ Vaginal Swab Transport Media (STM)[3]	Hologic™
Affirm Ambient Temperature Transport System	BD™
BD ProbeTec™ Swab diluent Q^x	BD™

[1] If samples appear dense or cloudy or have been stored >48 hours, see Appendix A, "Troubleshooting".

[2] Process samples within 48 hours of collection.

[3] Samples require a different DNA isolation procedure. See Appendix B, "Isolate DNA using the MagMAX™ DNA Multi-Sample Ultra Kit: Hologic™ Aptima™ media".

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 4 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

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.

- See the collection system or media documentation provided by the manufacturer for information on sample collection and storage.
- 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-well standard heat 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.

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 Buffer	1,000 µL
	3	Wash 2 Plate	80% Ethanol	1,000 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

The plate that the samples are concentrated in is the Sample Plate for the DNA isolation.

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 at 2,250 × *g* for 15 minutes to concentrate the samples.
4. After centrifugation, carefully remove and discard as much supernatant as possible without disturbing the pellet.

   Note: You can leave up to 100 µL of supernatant, especially if there is no pellet.

Set up the Sample Plate, then start processing

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 and the optional control of the KingFisher™ 96 Deep-Well Plate (Sample Plate).
3. On the KingFisher™ Flex instrument, select the **MVP_Ultra_Flex** script, then press **Start**.
4. Follow the instrument prompts to load sample and processing plates, then press **Start**.

Proceed immediately to the next section.

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
Total	**550 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 550 µL to each sample and control well in the Sample Plate.

   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 10.

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

"Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software" on page 23

▼

"Set up the PCR reactions in an OpenArray™ 384-well Sample Plate" on page 24

▼

"Set up the OpenArray™ AccuFill™ Instrument and the OpenArray™ AccuFill™ Software" on page 25

▼

"Transfer reactions to the OpenArray™ Plate using the OpenArray™ AccuFill™ Instrument" on page 27

▼

"Seal the OpenArray™ Plate" on page 39

▼

"Run the OpenArray™ Plate on the QuantStudio™ 12K Flex Instrument" on page 42

▼

"Check the quality–control images" on page 43

▼

Chapter 7, "Export and review vaginal microbiota profiling data"

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 28 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. To prepare for the next stage of the protocol, remove an OpenArray™ Plate from the freezer. 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 (which is created using the following steps).

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 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.

Component[1]	OpenArray™ Plate Format		
	18	56	112[2]
	Volume per well	Volume per well	Volume per well
TaqMan™ OpenArray™ Real-Time PCR Master Mix	2.5 µL	2.5 µL	2.5 µL
DNA sample	2.5 µL	2.5 µL	2.5 µL
Total reaction volume	**5.0 µL**	**5.0 µL**	**5.0 µL**

[1] *(Optional)* Include the TaqMan™ Vaginal Microbiota Amplification Control as described in the *TaqMan™ Vaginal Microbiota Amplification Control Product Information Sheet* (Pub. No. MAN0016007).

[2] For the 112-format, the OpenArray™ Sample Tracker Software designates two wells for each sample.

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.

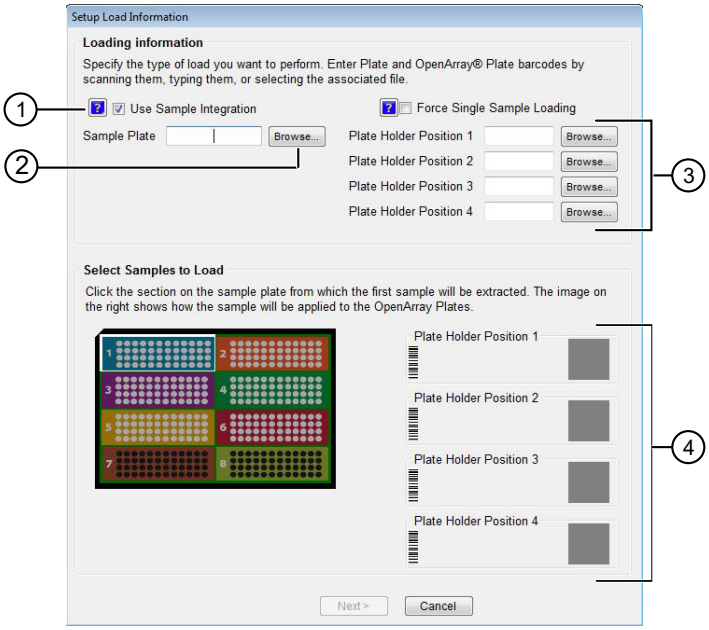
   Do not remove the foil from the scored area at this time.

If you make a sample layout error, generate 384-well sample plate layouts with a corrected 96-well sample CSV file (see “Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software” on page 23).

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 39.

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

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 28.

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.

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

For required materials, see “Required materials for the OpenArray™ Plate workflow” on page 10.

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

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. To prepare for the next stage of the protocol, remove an OpenArray™ Plate from the freezer. 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 (which is created using the following steps).

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.

Component[1]	OpenArray™ Plate Format		
	18	56	112[2]
	Volume per well	Volume per well	Volume per well
TaqMan™ OpenArray™ Real-Time PCR Master Mix	2.5 µL	2.5 µL	2.5 µL
DNA sample	2.5 µL	2.5 µL	2.5 µL
Total reaction volume	**5.0 µL**	**5.0 µL**	**5.0 µL**

[1] *(Optional)* Include the TaqMan™ Vaginal Microbiota Amplification Control as described in the *TaqMan™ Vaginal Microbiota Amplification Control Product Information Sheet* (Pub. No. MAN0016007).

[2] For the 112-format, the software designates two wells for each sample.

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 a 384-well plate (see "Set up the PCR reactions in an OpenArray™ 384-well Sample Plate (OpenArray™ AccuFill™ Software v2.0)" on page 32).
- Place the sample plate in the sample plate holder on the 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 materials in the QuantStudio™ 12K Flex OpenArray™ Accessories Kit
  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 34.

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.

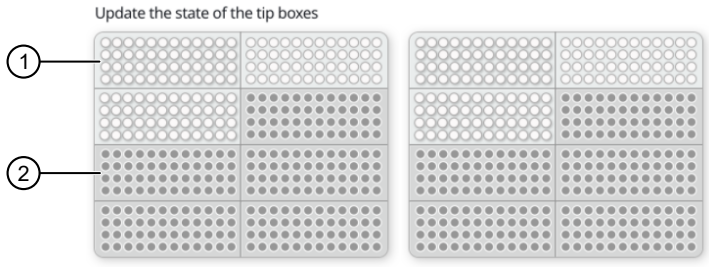
If the sample plate file was not imported, the samples must be added manually.

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

Proceed to "Verify the run setup and start the run" on page 34.

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**.

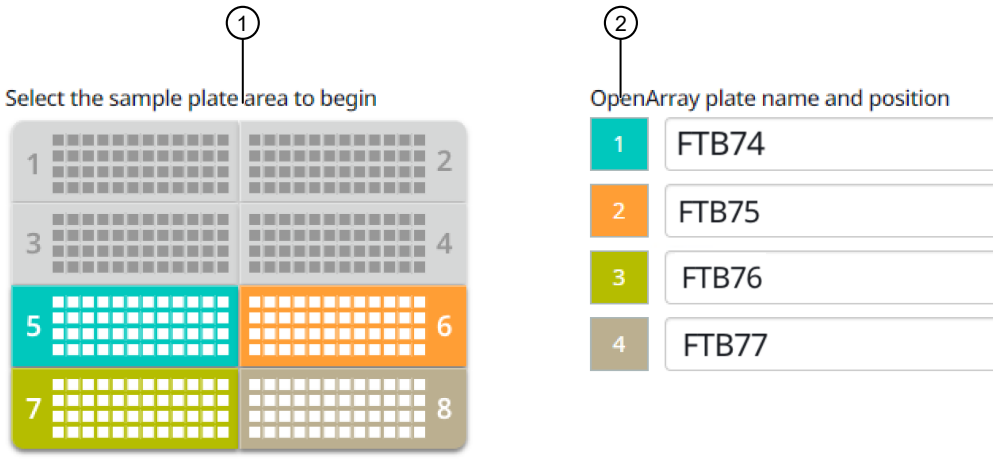
   The status of all section of the tips boxes is set to full.

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

   Select the first section of the sample plate if multiple OpenArray™ Plates are filled during a run. The software selects the total number of sections that correspond with the total number of OpenArray™ 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 OpenArray™ 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 OpenArray™ 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 the following conditions:

   - The waste bin is not in position
   - The sample plate is not in position
   - The OpenArray™ Plates are not in position
   - There are more OpenArray™ Plates on the instrument deck than are defined in the experiment setup
   - The instrument door is open

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 36).

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).

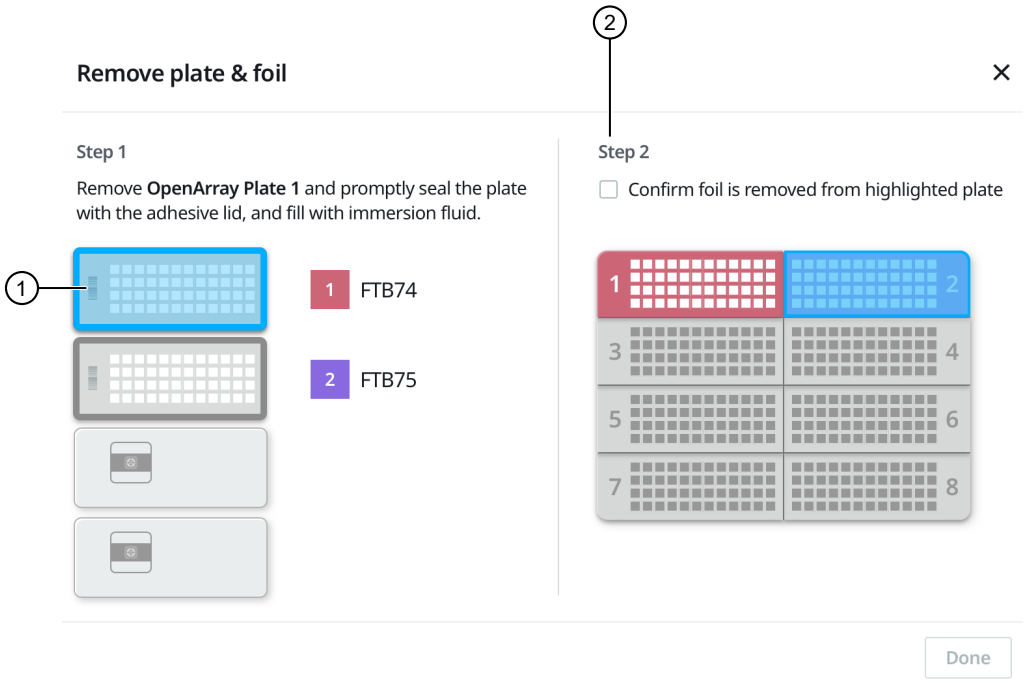


Figure 1 Remove plate and foil dialog box.

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).

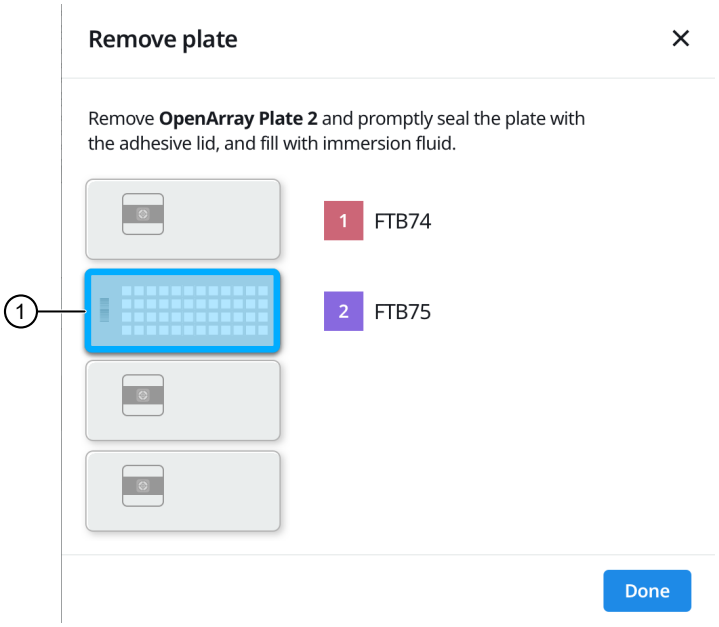


Figure 2 Remove plate dialog box

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 39.

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.

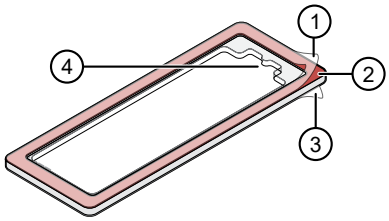
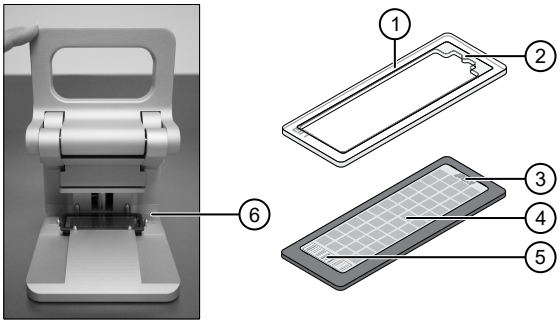


Figure 3 OpenArray™ Lid

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.

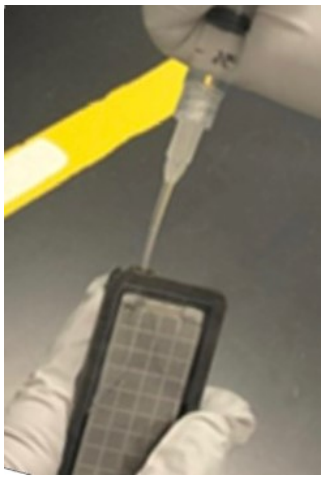
Note: Do not apply additional pressure onto the Plate Press during its actuation.

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

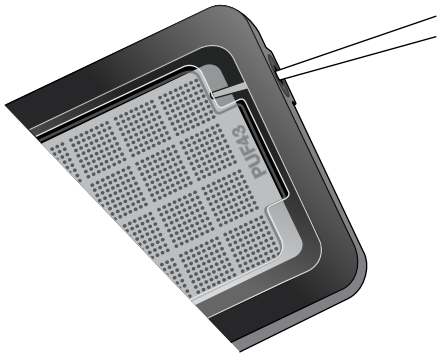
7. Prepare the immersion fluid. Remove the cap, insert the accompanying syringe tip, and prime the syringe by ejecting a small amount of immersion fluid onto a paper towel to ensure no air gap remains in the newly attached pipette tip.

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.



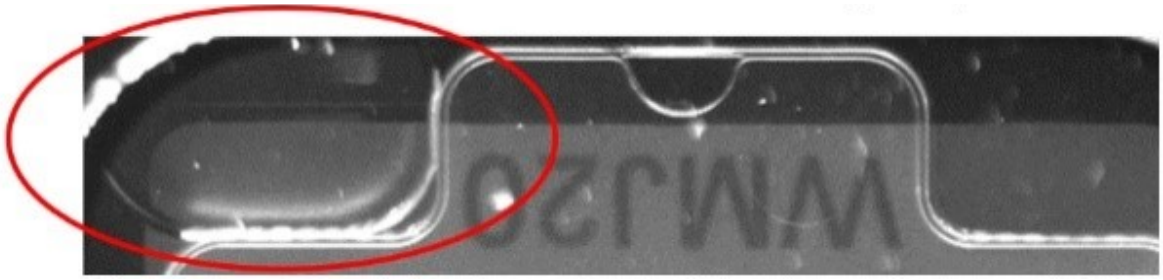
The syringe tip must be in front of the array when filling the case with immersion fluid.



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 vaginal 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 **Note:** Possible exceptions could include: • 16s rRNA (Ba04930791_s1) — acceptable range is 0.7 –1.0 • RNase P (Hs04930436_g1) — acceptable range is 0.7 –1.0
Amp Score	Amp Score < 1.24 **Note:** Possible exceptions could include: • *G. vaginalis* (Ba04646236_s1) — acceptable range is 1.1 –1.6

Note: Through-holes with unexpected C_{rt} values can also be identified by reviewing the Amplification Plot (see page 47).

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} ≤ 25 and a high standard deviation (> 0.5). The data from these through-holes may 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.

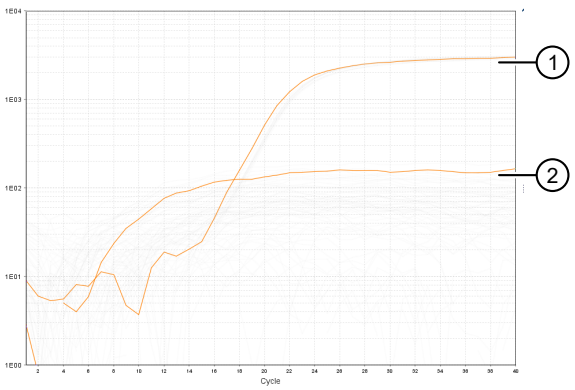
A

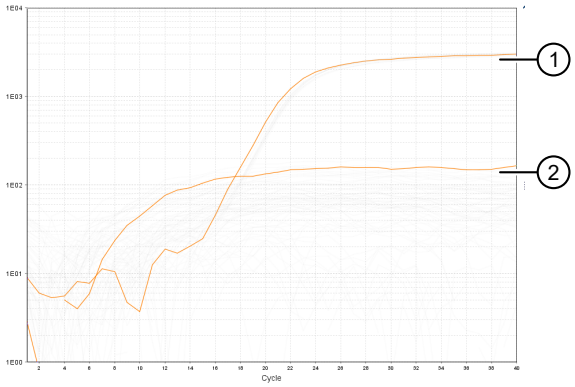
Troubleshooting

Troubleshoot DNA isolation errors

Observation	Possible cause	Recommended action
ESwab™ samples appear cloudy or dense	Samples have been stored for more than 48 hours (at room temperature or lower). **Note:** With extended storage, samples become more viscous which can cause the DNA Binding Beads to clump. DNA recovery may be reduced, impacting downstream performance.	Modify the DNA isolation protocol to improve DNA recovery: 1. Reduce the sample volume to 150–200 µL in "Concentrate the samples" on page 20. 2. Increase the volume of both DNA Elution Buffers to 50 µL in "Set up the processing plates" on page 58.

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.

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) *(continued)*	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.

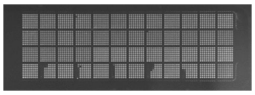
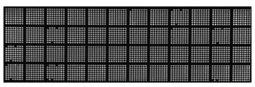
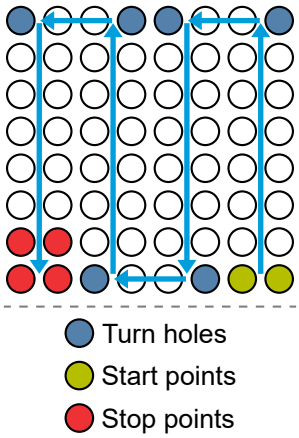
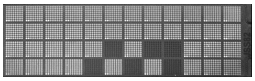
2. Use ImageJ to view the images of interest.

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.

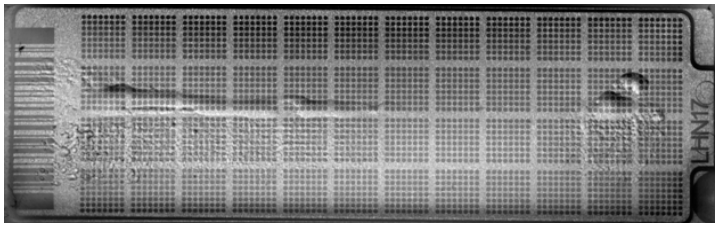
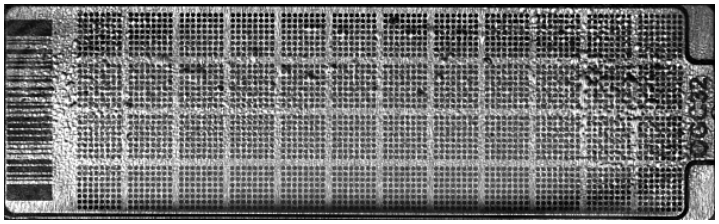
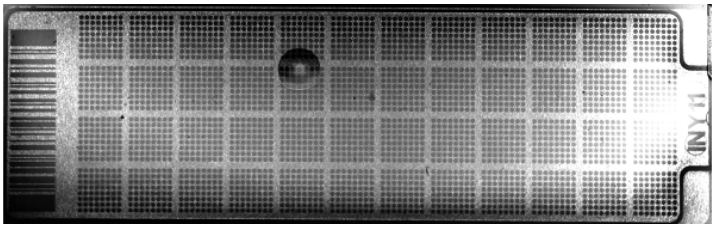
[1] The “cp#” in the image file name refers to the array position (1–4) within the QuantStudio™ 12K Flex Real-Time PCR Instrument.

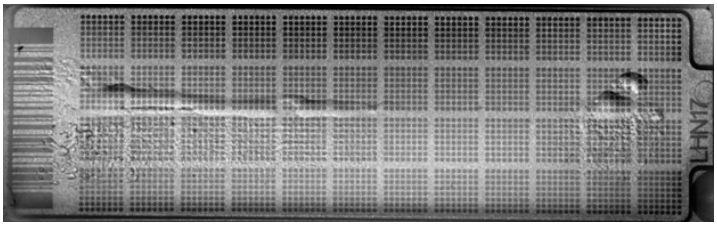
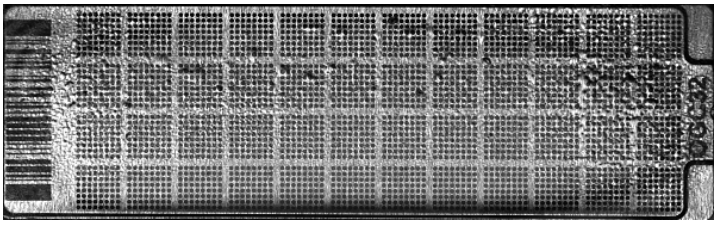
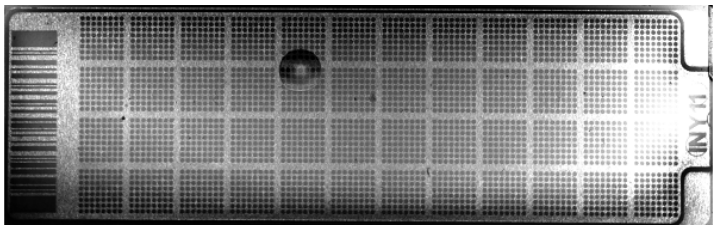
3. *(Optional)* Adjust the images for brightness and/or contrast to make image features visible.
   a. Open the image in ImageJ.
   b. Select **Image** ▸ **Adjust Brightness/Contrast** (or press **Ctrl+Shift+C**).
   c. Click **Auto** or adjust the sliders until the features of interest in the image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

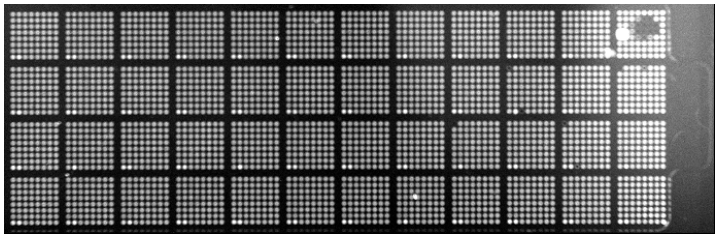
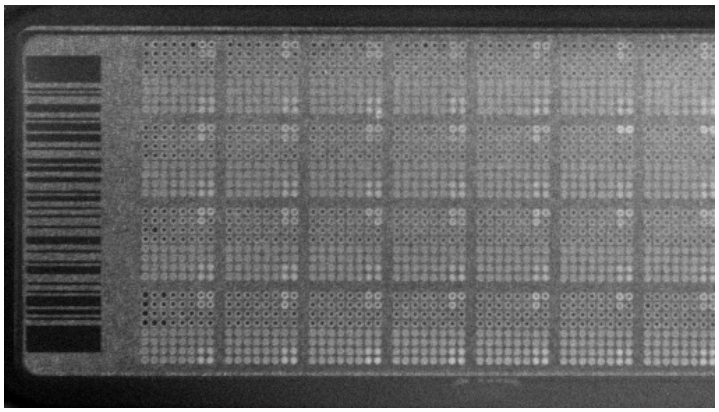
OpenArray™ AccuFill™ Instrument plate loading errors

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.
	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 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 49.	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
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 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 49. *(continued)*	Too large of a bubble inside the OpenArray™ Case after sealing. Damaged plate press, leading to uneven pressure.	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. Contact your field service engineer if you suspect that your plate press may be damaged.

Observation	Possible cause	Recommended action
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

Isolate DNA using the MagMAX™ DNA Multi-Sample Ultra Kit: Hologic™ Aptima™ media

This section describes instructions specific to samples collected using the Hologic™ Aptima™ Vaginal Swab Transport Media (STM). This protocol uses the MagMAX™ DNA Multi-Sample Ultra Kit.

See the collection system or media documentation provided by the manufacturer for information on sample collection and storage.

Contents and storage

Table 5 MagMAX™ DNA Multi-Sample Ultra Kit

Contents	Cat. No. A25597[1]	Cat. No. A25598[2]	Storage
Proteinase K	4 mL	5 × 4 mL	–25°C to –15°C
PK Buffer	96 mL	5 × 96 mL	15°C to 30°C
Multi-Sample DNA Lysis Buffer	100 mL	5 × 100 mL	
DNA Binding Beads	8 mL	5 × 8 mL	2°C to 8°C
RNase A	2 × 1.25 mL	10 × 1.25 mL	–25°C to –15°C
Nuclease-free Water	100 mL	5 × 100 mL	15°C to 30°C

Table 5 MagMAX DNA Multi-Sample Ultra Kit *(continued)*

Contents	Cat. No. A25597[1]	Cat. No. A25598[2]	Storage
Wash Solution 1 Concentrate[3]	80 mL	5 × 80 mL	15°C to 30°C
Wash Solution 2 Concentrate[3]	162 mL	5 × 162 mL	
DNA Elution Buffer 1	25 mL	5 × 25 mL	
DNA Elution Buffer 2	25 mL	5 × 25 mL	

[1] 500 reactions.
[2] 2,500 reactions.
[3] Before use of the kit, prepare all applicable wash solutions as described on their bottles and in this protocol.

Required materials not supplied

For other required materials, see “Required materials not supplied” on page 8.

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.

Table 6 Additional materials and equipment required for processing vaginal samples with Hologic™ Aptima™ media

Item	Source
B-PER™ Bacterial Protein Extraction Reagent	78243
Lysozyme Solution	90082
Zymolyase	Fisher Scientific 50-444-504

Download the KingFisher™ Flex program (if needed)

The program required for this protocol is not pre-installed on the KingFisher™ Flex Magnetic Particle Processor.

1. On the MagMAX™ DNA Multi-Sample Ultra Kit web page, scroll down to the **Product Literature** section.
2. Click **A25597_Vaginal** to download the program to your computer.
3. See *Thermo Scientific™ KingFisher™ Flex User Manual* (Cat. No. N07669) and *BindIt™ Software User Manual* (Cat. No. N07974) for instructions for installing the program on the instrument.

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.	

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.

- See the collection system or media documentation provided by the manufacturer for information on sample collection and storage.
- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Preheat an incubator to 65°C before each use of the kit.
- Use the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head and 96-well standard heat 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.
- For convenience, you can extend the Proteinase K digestion to 30 minutes.

Before first use of the kit

- Prepare the Wash Solutions from the concentrates:
  - Add 25 mL of isopropanol to Wash Solution 1 Concentrate, mix, then store at room temperature.
  - Add 132 mL of ethanol to Wash Solution 2 Concentrate, mix, then store at room temperature.
- Reconstitute the zymolyase with 500 µL of the provided storage buffer (final concentration of 4 U/µL), vortex to mix, then store at –20°C.

  For more information, see the documentation provided with the zymolyase.

Digest the samples with the Preliminary Digestion Mix

1. Prepare sufficient Preliminary Digestion Mix according to the following table.

   IMPORTANT! Prepare the Preliminary Digestion Mix no more than 30 minutes before use and store on ice. Prolonged storage at room temperature can reduce its efficiency.

Component	Volume per well	Volume per plate
B-PER™ Bacterial Protein Extraction Reagent	185 µL	18.5 mL
Lysozyme Solution	10 µL	1 mL
Zymolyase solution (4 U/µL)	5 µL	0.5 mL
Total Preliminary Digestion Mix	**200 µL**	**20 mL**

2. Invert the sample collection vial 5 times to ensure thorough mixing of the sample.
3. Following the sample layout, transfer 200 µL of sample to the appropriate wells of a deep-well plate.
4. Add 200 µL of Preliminary Digestion Mix to each sample well.
5. Seal the plate with a clear adhesive film, then shake at 1,050 rpm for 2 minutes.
6. Incubate the plate for 15 minutes at 65°C.

   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.

   During the incubation, prepare the PK Mix (next section).

Digest the samples with Proteinase K

1. Prepare sufficient PK Mix according to the following table, then invert several times to thoroughly mix components.

   IMPORTANT! Prepare the PK Mix no more than 30 minutes before use and store at room temperature. Do not place PK Buffer or PK Mix on ice, to avoid precipitation.

Component	Volume per well	Volume per plate
Proteinase K	8 µL	0.8 mL
PK Buffer	72 µL	7.2 mL
Total PK Mix	**80 µL**	**8.0 mL**

2. When the incubation with Preliminary Digestion Mix is complete, add 80 µL of PK Mix to each sample well of the plate.

3. Seal the plate with a clear adhesive film, then shake the sealed plate at 1,050 rpm for 3 minutes.

4. Incubate for 15 minutes at 65°C.

   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.

Set up the processing plates

1. While the samples are incubating at 65°C, set up the Wash, Elution, and Tip Comb Plates outside the instrument as described in the following table.

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Deep Well	Wash Solution 1	150 µL
Wash Plate 2	3	Deep Well	Wash Solution 2	150 µL
Wash Plate 3	4	Deep Well	Wash Solution 2	150 µL
Elution Plate[2]	5	Standard	DNA Elution Buffer 1	30 µL
Tip Comb	6	Deep Well	Place a tip comb in the plate.	

[1] Position on the instrument
[2] The instrument prompts the user to add DNA Elution Buffer 2 to the Elution Plate, after incubation with DNA Elution Buffer 1.

2. *(Optional)* To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.

Add Multi-Sample DNA Lysis Buffer, Bead/RNase A Mix, and isopropanol

1. *(Optional)* If condensation is present at the end of the 65°C incubation, briefly centrifuge the plate at 1,500 × *g* for 1–2 minutes.

2. Prepare sufficient Bead/RNase A Mix according to the following table.

   IMPORTANT! Prepare the Bead/RNase A Mix no more than 1 hour before use and store on ice. Prolonged storage at room temperature can reduce its efficiency.

   Vortex the DNA Binding Beads at moderate speed to form a uniform suspension before preparing the Bead/RNase A Mix.

Component	Volume per well	Volume per plate
DNA Binding Beads	16 µL	1.6 mL
RNase A	5 µL	0.5 mL
Nuclease-free Water	19 µL	1.9 mL
Total Bead/RNase A Mix	**40 µL**	**4.0 mL**

3. Add 200 µL of Multi-Sample DNA Lysis Buffer to each sample.

4. Vortex the Bead/RNase A Mix at moderate speed to ensure thorough mixing, then add 40 µL to each sample.

   If you see that the beads in the Bead/RNase A Mix are settling, vortex the mix again briefly before continuing to pipette.

5. Add 315 µL of isopropanol to each sample, then proceed immediately to process the samples on the instrument (next section).

Process samples on the instrument

1. Select the program on the instrument.
   - KingFisher™ Flex Magnetic Particle Processor: **A25597_Vaginal**
   - MagMAX™ Express-96 Magnetic Particle Processor: **4413021_DW_blood**

2. Start the run, remove the temporary paraffin plate seals (if present), then load the prepared processing plates in their positions when prompted by the instrument.

3. Load the sample plate (containing lysate, isopropanol, and Bead/RNase A Mix) at position 1 when prompted by the instrument.

4. When prompted by the instrument (approximately 25 minutes after initial start):
   a. Remove the Elution Plate from the instrument.

b. Add 30 µL of DNA Elution Buffer 2 to each sample well.

IMPORTANT! Add DNA Elution Buffer 2 immediately after the prompt, to prevent excessive drying of any beads that are still captured on the Tip Comb.

c. Load the Elution Plate back onto the instrument, and press **Start**.

5. At the end of the run (approximately 30 minutes after initial start), remove the Elution Plate from the instrument and seal immediately with a new clear adhesive film.
   - *(Optional)* Eluates can be transferred to a new storage plate after collection.
   - If you see excessive bead residue in the wells, place the Elution Plate on the Magnetic Stand-96 to capture any residue prior to downstream use of the DNA.

IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.

The purified samples are ready for immediate use. Alternatively, store the covered Elution Plate:

- At 2–6°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

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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)*, 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

Documentation and support

Related documentation

Document	Publication Number
MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit (automated extraction) User Guide	MAN0018075
OpenArray™ Vaginal Microbiota Profiling Experiments with OpenArray™ AccuFill™ Software v1.2 Quick Reference	MAN0015936
OpenArray™ Vaginal Microbiota Profiling Experiments with OpenArray™ AccuFill™ Software v2.0 Quick Reference	MAN0026017
TaqMan™ Vaginal Microbiota Amplification Control Product Information Sheet	MAN0016007
QuantStudio™ 12K Flex Real–Time PCR System OpenArray™ Vaginal Microbiota Starter Kit Quick Reference	MAN0016009
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935
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
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

Customer and technical support

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

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

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

Limited product warranty

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

Index

T

V

W