Respiratory Tract Microbiota Profiling Experiments v2

OpenArray™ Plates and OpenArray™ AccuFill™ Software v2.0

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Respiratory Tract Microbiota Profiling Experiments v2 using OpenArray™ Plates Application Guide* (Pub. No. MAN0019506). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This document is intended as a benchtop reference for experienced users. See "Related documentation" on page 7 for resources that contain detailed instructions and troubleshooting.

Note: This document includes instructions for OpenArray™ AccuFill™ Software v2.0. For instructions for OpenArray™ AccuFill™ Software v1.2, see *Respiratory Tract Microbiota Profiling Experiments v2 using OpenArray™ Plates and OpenArray™ AccuFill™ Software v1.2 Quick Reference* (Pub. No. MAN0019509).

There are multiple workflows available for OpenArray™ AccuFill™ Software v2.0. This document covers the full run workflow. For other workflows, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

Perform preamplification and dilute samples

Perform preamplification

Start with isolated nucleic acid. For the nucleic acid isolation procedure, see *Respiratory Tract Microbiota Profiling Experiments v2 using OpenArray™ Plates Application Guide* (Pub. No. MAN0019506).

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

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

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

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

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

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

3. Seal the plate with adhesive film.

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

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

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

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

6. Store the plate on ice until dilution for PCR (see "Dilute the preamplified sample" on page 2).

Dilute the preamplified sample

To determine dilution volumes, first determine the total volume of diluted preamplified sample that is required for PCR (see "Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0" on page 2). We recommend that you prepare only the volume of diluted preamplified sample that is required for your experiment. The undiluted preamplified sample can be stored at –20°C long term.

1. Vortex, then briefly centrifuge the plate that contains the completed preamplification sample reactions.
2. Remove the adhesive film from the plate.
3. Prepare a 1:10 dilution of the preamplified samples in a new 96-well plate.
   a. Transfer the desired volume of the preamplified samples to a new 96-well plate (for example, 2 µL).
   b. Add the appropriate volume of nuclease-free water to each sample and control well (for example, 18 µL).
4. Seal the plate with new adhesive film.
5. Vortex the plate for 10 seconds, then briefly centrifuge.
6. Proceed directly to prepare the OpenArray™ Plates (see "Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0" on page 2).

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

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

This section describes the procedures for a full run workflow for loading an OpenArray™ Plate on the OpenArray™ AccuFill™ Instrument with OpenArray™ AccuFill™ Software v2.0.

For instructions for OpenArray™ AccuFill™ Software v1.2 or for other workflow options, see "Related documentation" on page 7.

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	1. Enter the ***Lot number*** or ***Batch number***. 2. Enter one ***Serial number*** from the lot. **Note:** Only one serial number is required. The serial number is used to confirm the lot number or batch number. All of the files in the lot or batch are downloaded.
TaqMan OpenArray Inventoried	Enter the list of ***Serial numbers*** or ***Barcodes***. Separate more than one serial number or barcode with a comma or a line break. **Note:** The serial number or barcode entered corresponds to the file that is downloaded. Enter a serial number or barcode for each file to download.

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

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

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

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

The files are in a compressed ZIP folder.

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

Extract the files from the compressed ZIP folder.

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

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

1. Remove an OpenArray™ plate from the freezer and set it aside. Allow it to come to room temperature in its unopened sleeve (~15 minutes).

   The OpenArray™ plate must be completely thawed before transferring reactions to it from the OpenArray™ 384-well Sample Plate created in this section.

2. Gently swirl the contents of the TaqMan™ OpenArray™ Real-Time PCR Master Mix to thoroughly mix. Do not invert the bottle.

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

   (Optional) Use the TrueMark™ Respiratory Panel 2.0 Amplification Control in place of diluted preamplified sample, as a positive amplification control sample.

Component	Volume per well	Volume per sample
TaqMan™ OpenArray™ Real-Time PCR Master Mix	2.5 µL	5.0 µL
Diluted preamplified sample	2.5 µL	5.0 µL
Total reaction volume	**5.0 µL**	**10.0 µL**

   Note: An overage is recommended when preparing the components.

4. Thoroughly mix each PCR reaction by pipetting up and down or by using the "mix" function on a multi-channel pipette.

5. Seal the OpenArray™ 384-well Sample Plate with an aluminum foil seal, remove the foil flap, then mark the edges of the filled 4 × 12 area with a pen.

6. Centrifuge the plate at 1,200 × *g* for 1 minute.

7. Score the foil along the lines that were marked before centrifuging.

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

If you make a sample layout error, 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 2).

- 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, and 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 3.

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**.

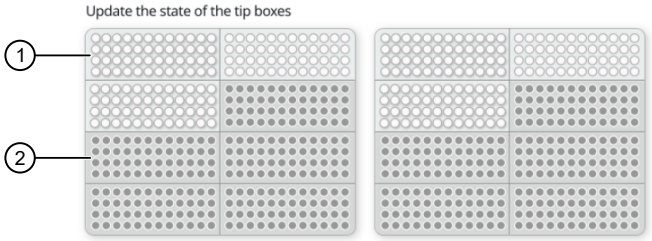
Proceed to "Verify the run setup and start the run" on page 4.

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**.

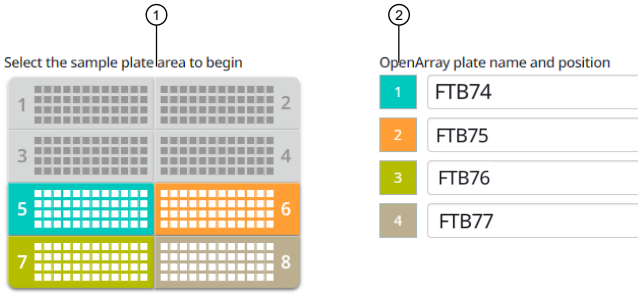
   The status of all section of the tips boxes is set to full.

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 4).

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 on page 4).

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 on page 5).

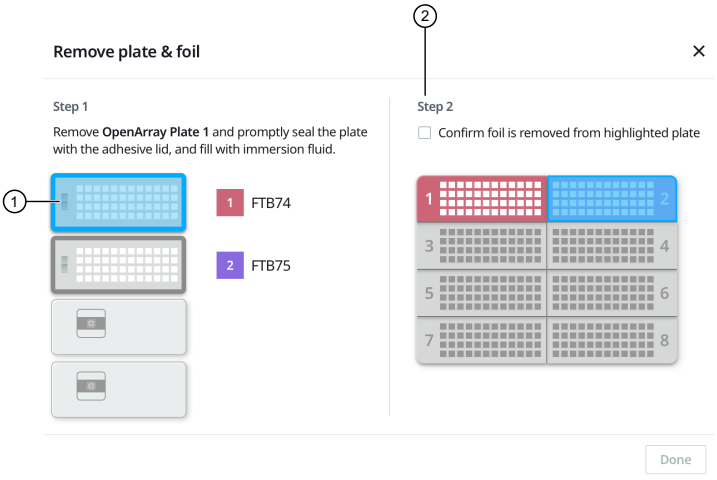


Figure 1 Remove plate and foil dialog box

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

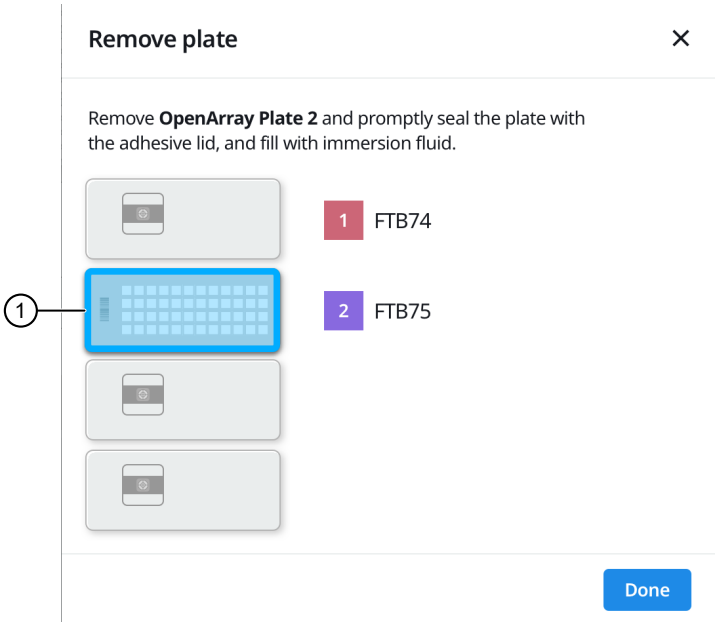


Figure 2 Remove plate dialog box

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 5.

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 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 instrument will proceed to load the next OpenArray™ Plate.

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

After all of the OpenArray™ 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).

Seal the OpenArray™ Plate

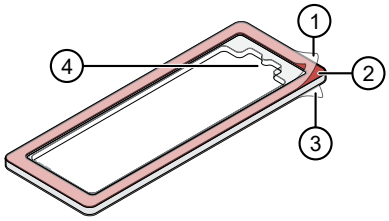
IMPORTANT! Throughout this procedure, handle the OpenArray™ Plate and the OpenArray™ Case only by the edges.

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

1. Place the newly loaded OpenArray™ Plate in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.

   Ensure that the barcode is facing left and the serial number is facing right.

2. From the OpenArray™ Lid, remove the clear protective film from the *inside* of the lid and the red adhesive-protective strip from around the edge of the lid.



OpenArray™ Lid

① Protective film on inside of the lid (remove before *sealing*)
② Red adhesive-protective strip (remove before *sealing*)
③ Protective film on the outside of the lid (remove before *running*)
④ Notched end (align with serial number on plate)

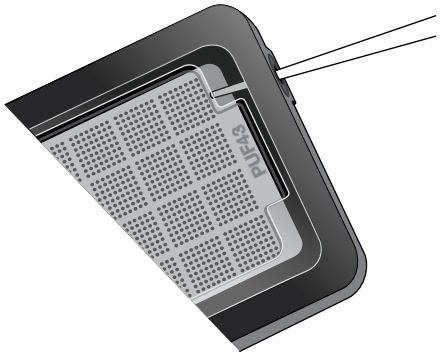
3. Seat the lid on the OpenArray™ Plate with the lid adhesive against the plate.

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

5. Disengage the press and remove the OpenArray™ Case.

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

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



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

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

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

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

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

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

1. On the QuantStudio™ 12K Flex Instrument touchscreen, touch to extend the instrument tray arm.

2. Remove the clear protective film from the outside of the OpenArray™ case (sealed plate + lid).

3. Place the OpenArray™ case on the tray arm plate adapter.
   - Support the case from underneath the tray arm to prevent the case from slipping through the adapter.
   - Ensure that the plate barcode and serial number are facing the front of the instrument.

4. Touch to retract the instrument tray arm.

5. In the **Home** screen of the QuantStudio™ 12K Flex Software, in the **Run** pane, click **OpenArray**.

6. In the **Select Instrument** pane, select your instrument.

7. Click **Get Plate IDs** to import the barcode of the OpenArray™ Plate.

   Once the OpenArray™ serial number appears, the loaded TPF file corresponding to the plate should appear in the **Setup File** field.

   If the TPF file does not appear, click **Browse**, then select the correct loaded TPF file from the **Loaded TPF** folder.

8. *(Optional)* Click **Browse** to change the **Experiment File Location**.

9. *(Optional)* Change the software-determined **Experiment File Name**.

10. Click **Start Run**.

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

11. Transfer the EDS file from the instrument to an accessible location for analysis.

12. Check the QC images for loading issues or leaks.

Check the quality–control images

Check the quality–control (QC) images before analysis. Images can be viewed using ImageJ, an open–source software available from the NIH at **imagej.nih.gov/ig**.

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.

Related documentation

Document	Pub. No.
Respiratory Tract Microbiota Profiling Experiments using OpenArray™ Plates Application Guide	MAN0017952
TaqMan™ OpenArray™ Respiratory Tract Microbiota Plate Product Information Sheet	MAN0018631
Respiratory Tract Microbiota Profiling Experiments using OpenArray™ Plates and OpenArray™ AccuFill™ Software v1.2 Quick Reference	MAN0018529
TaqMan™ Respiratory Tract Microbiota Amplification Control Product Information Sheet	MAN0018533
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935
OpenArray™ AccuFill™ System User Guide, for OpenArray™ AccuFill™ Software v1.2	MAN0025669
OpenArray™ AccuFill™ Software v2.0 Full Run Workflow Quick Reference	MAN0025836
OpenArray™ AccuFill™ Software v2.0 Quick Run Workflow Without Sample Information Quick Reference	MAN0025835

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

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Revision history: Pub. No. MAN0026009

Revision	Date	Description
A.0	6 December 2021	New document for the OpenArray™ Respiratory Tract Microbiota Profiling Experiments v2 with OpenArray™ AccuFill™ Software v2.0.

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