TaqMan™ Controls with TaqMan™ OpenArray™ Plates

Pub. No. MAN0025036 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

This document is intended as a benchtop reference for the Applied Biosystems™ TaqMan™ Comprehensive Microbiota Control and the Applied Biosystems™ TaqMan™ Custom DNA Control with TaqMan™ OpenArray™ Plates.

For detailed instructions to handle and prepare TaqMan™ OpenArray™ Plates, see the documents listed in “Related documentation” on page 7.

Product description

The TaqMan™ Comprehensive Microbiota Control and the TaqMan™ Custom DNA Control contain a linearized multi-target plasmid pool with microbial target sequences commonly used on TaqMan™ OpenArray™ panels and TaqMan™ Array Card panels.

The TaqMan™ Comprehensive Microbiota Control has targets relevant to many microbial profiling applications, including respiratory tract, urinary tract, vaginal microbiota, and antibiotic resistance research.

For a list of targets, go to: **https://www.thermofisher.com/order/catalog/product/A50382**.

The controls can be included as a positive control for panel-specific amplification. The high-concentration control can also be used to determine parameters necessary for analytical confirmation (e.g. limit of detection and C_t cut-off values).

The TaqMan™ Comprehensive Microbiota Control and the TaqMan™ Custom DNA Control also contain TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control and the human RNase P RPPH1 gene. Additionally, the TaqMan™ Comprehensive Microbiota Control carries a sequence specific to TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*).

During real-time PCR, the controls can be used as a stand-alone sample to verify assay performance and to help with troubleshooting.

Products are stable for one year from the date of manufacture when stored at –25°C to –15°C.

Required materials

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
TaqMan™ Comprehensive Microbiota Control	A50382 (1×10^5 copies/µL) A50383 (5×10^7 copies/µL)
TaqMan™ Custom DNA Control	(1×10^5 copies/µL) (5×10^7 copies/µL) Multiple targets available.[1]
No Template Control UltraPure™ DNase/RNase-Free Distilled Water	10977015
Centrifuge capable of spinning deep well plates at 200 × *g* or greater	MLS
Fisher Scientific™ Analog Vortex Mixer	Fisher Scientific™ 02-215-365
TE, pH 8.0, RNase-free	AM9849

Item	Source
PCR reaction mix preparation: Plates and film *or* tubes:	
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	4346906
MicroAmp™ Optical 96-Well Reaction Plate	4306737
MicroAmp™ Clear Adhesive Film	4306311
Nonstick, RNase-Free Microcentrifuge Tubes, 0.5 mL	AM12350
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	AM12450
Additional materials for OpenArray™ plate format	
TaqMan™ OpenArray™ Real-Time PCR Master Mix	4462164
OpenArray™ 384-Well Sample Plates (microplates), black	4482221
TaqMan™ OpenArray™ Plate	Custom ordered
OpenArray™ AccuFill™ System Tips	4458107
QuantStudio™ 12K Flex OpenArray™ Accessories Kit (contains the items needed to assemble up to 10 plates: 12 lids and plugs, 12 immersion fluid syringes, and 2 carriers)	4469576
OpenArray™ 384-Well Plate Seals	4469876
Instrument and software: • OpenArray™ Sample Tracker Software • QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0. • QuantStudio™ 12K Flex Instrument with OpenArray™ block (AccuFill™ System)	

[1] For a complete list of catalog numbers, see *TaqMan™ Custom DNA Controls Product Information Sheet* (Pub. No. MAN0024963).

Guidelines

- Before first use, thaw the control, vortex to thoroughly mix the contents, then centrifuge briefly to collect the contents at the bottom of the tube
- Create aliquots to reduce the number of freeze-thaw cycles.
- Do not exceed three freeze-thaw cycles.
- Aliquots are stable for four months when stored at 4°C, if the products are still within one year of the manufacture date. Storage at 4°C can prevent degradation from freeze-thaw cycles.

Prepare and run the TaqMan™ Controls

Dilute the controls

The TaqMan™ Control at 5×10^7 copies/µL is diluted to a final concentration of 1×10^5 copies/µL before use.

The TaqMan™ Control at 1×10^5 copies/µL can be used in the reaction.

1. Thoroughly thaw the TaqMan™ Control.
2. Vortex the tube to mix.
3. In a nonstick RNase-free microcentrifuge tube or a 96-well reaction plate, dilute the control (5×10^7 copies/µL) in a serial dilution to a concentration of 1×10^5 copies/µL, according to the tables below.

Component	Volume
Control at 5×10^7 copies/µL	10 µL
TE Buffer	40 µL
Total volume of control at 1×10^7 copies/µL	**50 µL**

Component	Volume
Control at 1 × 10^7 copies/µL (from previous table)	10 µL
TE Buffer	90 µL
Total volume of control at 1 × 10^6 copies/µL	**100 µL**

Component	Volume
Control at 1 × 10^6 copies/µL (from previous table)	10 µL
TE Buffer	90 µL
Total volume of control at 1 × 10^5 copies/µL	**100 µL**

4. Seal the plate with MicroAmp™ Clear Adhesive Film or cap the tubes.
5. Vortex to ensure thorough mixing, then centrifuge.

Prepare the control reactions

Dilute the TaqMan™ Control if it is at a concentration of 5 × 10^7 copies/µL (see "Dilute the controls" on page 2).

If the TaqMan™ Control is at a concentration of 1 × 10^5 copies/µL, it can be used in the reaction without a dilution step. Thaw the control thoroughly, then vortex the tube to mix.

1. Vortex the TaqMan™ OpenArray™ Real-Time PCR Master Mix gently.

   Note: Do not invert the bottle.

2. Combine the following reagents into a non-stick RNase-free microcentrifuge tube for each TaqMan™ OpenArray™ Plate.

Component	Volume[1]
TaqMan™ OpenArray™ Real-Time PCR Master Mix	2.75 µL
TaqMan™ Control (1 × 10^5 copies/µL)[2]	1.10 µL
UltraPure™ DNase/RNase-Free Distilled Water	1.65 µL
Total reaction volume	**5.5 µL**

[1] Volumes include10% overage.
[2] Use UltraPure™ DNase/RNase-Free Distilled Water for No-Template Controls.

3. Vortex to mix.
4. Add 5 µL into each well of a 384-well OpenArray™ plate.
5. Seal the plate with an aluminum foil seal (OpenArray™ 384-Well Plate Seals), remove the foil flap, mark the edges of the filled 4 × 12 area with a pen, then score the foil along those lines. Do not remove the foil from the scored area at this time.
6. Vortex the sealed plate for 5 seconds, then centrifuge the plate for 1 minute at 1,000 rpm. Do not vortex at too vigorous a setting, to avoid excessive bubble formation.

Set up the AccuFill™ instrument and software

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

1. In the OpenArray™ AccuFill™ software, click **Setup and Load**.

   The **Setup Load Information** window appears.

2. Configure the **Loading Information** pane for sample integration using the 384-well sample plate CSV file and TPF files.
   a. In the **Loading Information** pane (top section of the window), ensure that the **Use Sample Integration** checkbox is selected.
   b. Click **Browse** to the right of the **Sample Plate** field, then select the 384-well sample plate CSV file that you generated with the OpenArray™ Sample Tracker Software in the Sample Tracker 384-well CSV Files folder.

c. Click **Browse** to the right of the **Plate Holder Position** of the OpenArray™ plate, then select the TPF file for the OpenArray™ plate in the TPF Files folder.

3. In the **Select Samples to Load** pane (bottom section of the window), click the corresponding 4 × 12 area of the 384-well sample plate image, then click **Next**.

   The **Setup Deck** window is displayed.

4. In the AccuFill™ instrument, ensure that:
   - Tip boxes and tips are loaded as shown in the **Setup Deck** window.
   - The lids are removed from the tip boxes.
   - The waste bin in the instrument is emptied.

5. In the **Setup Deck** window, confirm that the deck is ready:
   - Select **The tips are configured as shown above**.
   - Select **The Waste Bin is empty**.

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

1. Prepare the items needed to seal the loaded OpenArray™ plate .

   Note: The OpenArray™ plate must be sealed promptly after being loaded with the reactions, as described here.
   - Ensure that the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0 is ready.
   - Gather and remove from their packaging the following: an OpenArray™ Lid, plug, syringe with OpenArray™ Immersion Fluid, and syringe tip.
   - Attach the syringe tip to the syringe, carefully push some of the fluid through the tip to remove air bubbles, then lay the syringe aside.

2. Load the OpenArray™ plate and the OpenArray™ 384-well Sample Plate into the AccuFill™ instrument.
   - **OpenArray™ plate**—Remove the plate from its sleeve, then place the plate in the appropriate plate holder position in the instrument.
     Ensure that the barcode on the OpenArray™ plate is facing left and the serial number is facing right.
   - **OpenArray™ 384-well Sample Plate**—Place the 384-well sample plate onto the deck of the instrument, then use forceps to peel the foil from the filled area of the plate.

3. Close the door of the AccuFill™ instrument.

4. In the AccuFill™ software **Setup Deck** window, select the following confirmations:
   - **The OpenArray Plate is in the Plate Holder**
   - **Remove foil from the highlighted section of the Sample Plate**

5. Click **Load**.

6. As soon as the **Remove OpenArray Plate** window appears, open the instrument door and remove the loaded OpenArray™ plate.

7. Proceed immediately to seal the OpenArray™ plate .

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

Seal the OpenArray™ plate

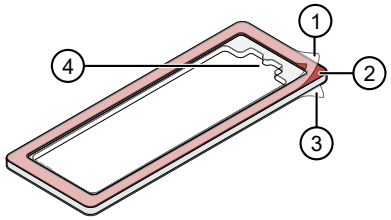
IMPORTANT! Throughout this procedure, handle the OpenArray™ plate and the OpenArray™ Case only by the edges.

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

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

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

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

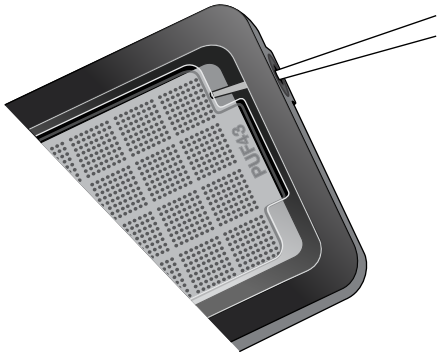


OpenArray™ Lid

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

3. Seat the lid on the OpenArray™ plate with the lid adhesive against the plate.
4. Engage the press mechanism until the green flashing light changes to a steady green light (approximately 20 seconds).
5. Disengage the press and remove the OpenArray™ Case.
6. While holding the case by its edges, insert the prepared syringe tip into the port in the case, then carefully inject OpenArray™ Immersion Fluid until the case is filled.

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



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

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

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

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

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

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

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.

Related documentation

Document	Publication number
TaqMan™ Comprehensive Microbiota Control Product Information Sheet	MAN0024964
TaqMan™ Custom DNA Controls Product Information Sheet	MAN0024963
Respiratory Tract Microbiota Profiling Experiments using OpenArray™ Application Guide	MAN0017952
Respiratory Tract Microbiota Profiling Experiments v2 using OpenArray™ Application Guide	MAN0019506
Urinary Tract Microbiota Profiling Experiments Application Guide	MAN0017750
Vaginal Microbiota Profiling Experiments Application Guide	MAN0015669

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

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Thermo Fisher Scientific GENEART GmbH | Im Gewerbepark B35 | 93059 Regensburg | Germany
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Revision	Date	Description
A.0	23 April 2021	New document for the TaqMan™ Comprehensive Microbiota Controls and the TaqMan™ Custom DNA Controls with TaqMan™ OpenArray™ Plates.

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