TrueMark™ Wound Microbiota and Antibiotic Resistance Gene Profiling Experiment—OpenArray™ Plate format

USER GUIDE

TaqMan™ Assays for wound microbiota and antibiotic resistance gene profiling experiments using OpenArray™ Plates

for use with:

TrueMark™ OpenArray™ RT PCR Custom Format, Study Name "Wound+ABR Expanded Panel"

Custom TrueMark™ OpenArray™ Plate

MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit

MagMAX™ Prime Viral/Pathogen NA Isolation Kit

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

Catalog Number 4471120

Publication Number MAN0029872

Revision B.0

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

Revision history: MAN0029872 B.0 (English)

Revision	Date	Description
B.0	8 February 2024	• Updated the list of assays. • Included instructions for EDT files in Chapter 3, "Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0". • Removed instructions for OpenArray™ AccuFill™ Software v1.2. • Updated to TrueMark™ branding.
A.0	21 August 2023	New user guide for TrueMark™ Wound Microbiota and Antibiotic Resistance Gene Profiling Experiment—OpenArray™ Plate format.

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

Contents

1 Overview

Product description

TrueMark™ OpenArray™ RT PCR Custom Format, Study Name "Wound+ABR Expanded Panel" (Cat. No. 4471120, Array ID MA47VXD) is an efficient, easy-to-use fixed-content OpenArray™ Plate for the characterization of key wound microbiota targets and antibiotic resistance gene families. The plate includes TaqMan™ assays that have been optimized for detection of bacteria, fungi, and antibiotic resistance gene targets. The plate also includes control assays for TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*) and TrueMark™ Xeno Control, Kanamycin Resistance. For a complete list of assays included in the plate, see “TaqMan™ assays for wound microbiota and antibiotic resistance gene targets” on page 7.

The assays perform well with total nucleic acid that is isolated from wound swabs using the MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit or MagMAX™ Prime Viral/Pathogen NA Isolation Kit.

TaqMan™ assay designs and assay target sequences have undergone rigorous bioinformatics selection and analysis to maximize strain coverage and minimize potential for off-target cross-reactivity. Qualified TaqMan™ assays have undergone performance testing to ensure that results are accurate with high levels of sensitivity and specificity.

TaqMan™ assays for wound microbiota and antibiotic resistance gene targets

The following tables list assays that are included in the TrueMark™ OpenArray™ RT PCR Custom Format, Study Name "Wound+ABR Expanded Panel".

Table 1 Assays for wound microbiota targets

#	Pathogen	Bacterial gram strain	Assay ID
1	*Acinetobacter baumannii*	Gram-negative	Ba04932084_s1
2	*Bacteroides fragilis*	Gram-negative	Ba04646225_s1
3	*Candida auris*	N/A	Fn07921934_s1
4	*Candida albicans*	N/A	Fn00005034_po[1]
	Candida parapsilosis		
	Candida tropicalis		
5	*Candida glabrata*	N/A	Fn07922856_po[1]
	Candida krusei		
6	*Citrobacter braakii*	Gram-negative	Ba07922309_po[1]
	Citrobacter freundii	Gram-negative	
	Citrobacter koseri	Gram-negative	
7	*Clostridium perfringens*	Gram-positive	Ba07922559_s1
8	*Clostridium histolyticum*	Gram-positive	Ba07922644_po[1]
	Clostridium novyi A,B	Gram-positive	
	Clostridium septicum	Gram-positive	
	Clostridium sordellii	Gram-positive	
9	*Corynebacterium striatum*	Gram-positive	Ba07921944_s1
10	*Enterobacter cloacae*	Gram-negative	Ba04932087_s1
11	*Enterococcus faecalis*	Gram-positive	Ba04646247_s1
12	*Enterococus faecium*	Gram-positive	Ba04932086_s1
13	*Escherichia coli*	Gram-negative	Ba04646242_s1
14	*Finegoldia magna*	Gram-positive	Ba07921950_s1
15	*Klebsiella aerogenes*	Gram-negative	Ba04932080_s1
16	*Klebsiella oxytoca*	Gram-negative	Ba00005014_po[1]

Table 1 Assays for wound microbiota targets *(continued)*

#	Pathogen	Bacterial gram strain	Assay ID
16	*Klebsiella pneumoniae*	Gram-negative	Ba00005014_po[1]
17	*Morganella morganii*	Gram-negative	Ba04932078_s1
18	*Peptoniphilus asaccharolyticus*	Gram-positive	Ba07922642_po[1]
	Peptoniphilus harei	Gram-positive	
	Peptoniphilus ivorii	Gram-positive	
19	*Peptostreptococcus anaerobius*	Gram-positive	Ba07921938_s1
20	*Proteus mirabilis*	Gram-negative	Ba00005020_po[1]
	Proteus vulgaris	Gram-negative	
21	*Providencia stuartii*	Gram-negative	Ba04932077_s1
22	*Pseudomonas aeruginosa*	Gram-negative	Ba04932081_s1
23	*Serratia marcescens*	Gram-negative	Ba07921916_s1
24	*Staphylococcus aureus*	Gram-positive	Ba04646259_s1
25	*Staphylococcus epidermidis*	Gram-positive	Ba07922645_po[1]
	Staphylococcus haemolyticus	Gram-positive	
26	*Stenotrophomonas maltophilia*	Gram-negative	Ba07922562_s1
27	*Staphylococcus lugdunensis*	Gram-positive	Ba07921980_s1
28	*Streptococcus anginosus*	Gram-positive	Ba07922557_s1
29	*Streptococcus agalactiae*	Gram-positive	Ba07922641_po[1]
	Streptococcus dysgalactiae	Gram-positive	
30	*Streptococcus pyogenes*	Gram-positive	Ba07921919_s1

[1] To order the correct pool, use assay IDs listed in this table. For component assay IDs within each pool, see Table 9 on page 54.

Table 2 Assays for antibiotic resistance targets

#	Antibiotic	Gene family	Assay ID
1	AmpC beta-lactamase 1	blaACC	Ba07922649_po[1]
		blaFOX	
2	AmpC beta-lactamase 2	blaACT	Ba07922650_po[1]
		blaACT/blaMIR	
3	AmpC beta-lactamase 3	blaCMY/blaLAT	Ba00005013_po[1]

Table 2 Assays for antibiotic resistance targets *(continued)*

#	Antibiotic	Gene family	Assay ID
3	AmpC beta-lactamase 3	blaDHA	Ba00005013_po[1]
		blaMOX/blaCMY	
4	Beta-Lactamase	blaTEM	Ba04646128_s1
5	Carbapenemase 1	blaIMP	Ba07922413_po[1]
6	Carbapenemase 2	blaOXA-1	Ba07922647_po[1]
		blaOXA-2	
		blaOXA-23	
		blaOXA-51	
7	Carbapenemase 3	blaKPC	Ba07922648_po[1]
		blaNDM	
		blaOXA-48	
		blaVIM	
8	Extended-spectrum beta-lactamases 1	blaCTX-M	Ba07922646_po[1]
9	Extended-spectrum beta-lactamases 2	blaSHV	Ba04646134_s1
10	Extended-spectrum beta-lactamases 3	blaGES	Ba00005007_po[1]
		blaPER	
		blaVEB	
11	Lincosamide, Macrolide, Streptogramin	cfr	Ba07319992_s1
12	Macrolides 1	msr(A)	Ba07922570_s
13	Macrolides 2	ere(B)	Ba07922652_po[1]
		mef(A)	
		mph(A)	
14	Macrolides 3	erm(A)	Ba07922653_po[1]
		erm(B)	
		erm(C)	
15	Methicillin	mecA	Ba07922654_po[1]
		mecC	
16	Nitromidazole	nimB	Ba07922657_po[1]

Table 2 Assays for antibiotic resistance targets *(continued)*

#	Antibiotic	Gene family	Assay ID
16	Nitromidazole	nimD	Ba07922657_po[1]
		nimE	
		nimJ	
17	Quinolone 1	qnrB	Ba07922658_po[1]
18	Quinolone 2	qnrA	Ba07922659_po[1]
		qnrS	
19	Sulfonamide	sul1	Ba00005027_po[1]
		sul2	
20	Tetracycline 1	tet(M)	Ba04230915_s1
21	Tetracycline 2	tet(A)	Ba07922656_po[1]
		tet(B)	
		tet(S)	
22	Trimethoprim 1	dfrA1	Ba07922651_po[1]
		dfrA5	
		dfrA12	
		dfrA17	
23	Trimethoprim 2	dfrA14	Ba07922660_po[1]
		dfrB1	
		dfrB5	
		dfrG	
24	Vancomycin	vanA	Ba00005023_po[1]
		vanB	

[1] To order the correct pool, use assay IDs listed in this table. For component assay IDs within each pool, see Table 10 on page 55.

Table 3 Control assays

Control name	Assay name	Nucleic acid type	Assay ID
TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*)	B.atrophaeus	DNA	Ba06596576_s1
TrueMark™ Xeno Control, Kanamycin Resistance	Xeno	DNA	Pa00010014_a1

TrueMark™ OpenArray™ Plate products and formats

TrueMark™ OpenArray™ RT PCR Custom Format, Study Name "Wound+ABR Expanded Panel"

The TrueMark™ OpenArray™ RT PCR Custom Format, Study Name "Wound+ABR Expanded Panel" (Cat. No. 4471120, Array ID MA47VXD) contains pre-plated, dried down TaqMan™ assays for wound microbiota and antibiotic resistance gene profiling. For the complete lists of assays included with the plate, see “TaqMan™ assays for wound microbiota and antibiotic resistance gene targets” on page 7.

Contents and storage

Table 4 TrueMark™ OpenArray™ RT PCR Custom Format, Study Name "Wound+ABR Expanded Panel" (Cat. No. 4471120, Array ID MA47VXD)

Component	Amount	Array format	Storage
TrueMark™ OpenArray™ RT PCR Custom Format, Study Name "Wound+ABR Expanded Panel"	10 plates	56	–25°C to –15°C

The panel includes assays to the 44 microbial targets, 58 antibiotic resistance gene targets (a total of 54 unique assays), and 2 controls (TrueMark™ Xeno Control, Kanamycin Resistance and TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*)). Each TrueMark™ OpenArray™ RT PCR Custom Format, Study Name "Wound+ABR Expanded Panel" can be used to run up to 46 samples and 2 controls.

Custom TrueMark™ OpenArray™ Plate formats

Custom TrueMark™ OpenArray™ Plate contain pre-plated, dried down TaqMan™ assays for wound microbiota and antibiotic resistance gene profiling. Custom layouts can be configured using Assay IDs listed in “TaqMan™ assays for wound microbiota and antibiotic resistance gene targets” on page 7, along with other TaqMan™ microbe detection assays.

Array format	Number of assays	Maximum number of samples
56	56	48
112	112	24
168	168	16
224	224	12

Note: We recommend two or more technical replicates of each reaction.

Configure and order Custom TrueMark™ OpenArray™ Plates

1. Go to **https://www.thermofisher.com/microbe-detection/taqman/target-list/choose-format**.
2. For the format, select **OpenArray**.

3. Select your assay design as follows, then click **Next step**:
   - To open an existing Microbe OpenArray™ design, enter your Array ID.
   - For a new layout, click **Select** to configure a plate with the desired array format.

   Note: Specify the **Replicates of assays** you need.
   (Optional) In the table, click **View Layout** to preview the layout of the plate.
4. Enter the list of targets or import an existing list, then click **Submit**. When complete, click **Next step**.
5. *(Optional)* To add additional targets, click **Add another**. When complete, click **Next step**.
6. Follow the on-screen instructions to configure the assays on the plate.
7. *(Optional)* Click **Save progress** 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.

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.

Materials required for nucleic acid isolation

Nucleic acid isolation kit

Nucleic acid isolation can be performed using the MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit (Cat. No. A42356) or MagMAX™ Prime Viral/Pathogen NA Isolation Kit (Cat. No. A58145). For MagMAX™ Prime Viral/Pathogen NA Isolation Kit contents and storage information, see the *MagMAX™ Prime Viral/Pathogen NA Isolation Kit and Accessories User Guide* ("Related documentation" on page 61).

Table 5 MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit (Cat. No. A42356)

Component	Amount	Storage
Binding Solution	53 mL	15–25°C
Wash Buffer	100 mL	
Elution Solution	10 mL	
Total Nucleic Acid Binding Beads	2 mL	

Table 5 MagMAX Viral/Pathogen Ultra Nucleic Acid Isolation Kit (Cat. No. A42356) *(continued)*

Component	Amount	Storage
Proteinase K	1 mL	15–25°C
Enzyme Mix	5 mL	–25°C to –15°C

Additional materials required for nucleic acid isolation

Item	Source
Instrument	
Magnetic particle processor (one of the following, depending on quantity/volume of sample to be processed):	
For standard volume sample[1]: KingFisher™ Flex Purification System, KingFisher™ with 96 Deep-well Head	5400630
For large volume sample[2]: KingFisher™ Flex Purification System, KingFisher™ with 24 Deep-well Head	5400640
For standard volume sample[1]: KingFisher™ Apex with 96 Deep Well Head	5400930
For large volume sample[2]: KingFisher™ Apex with 24 Combi head	5400940
KingFisher™ Duo Prime Purification System	5400110
Consumables	
Deep-well plates:	
For standard volume sample[1]: KingFisher™ Deepwell 96 Plate, V-bottom, polypropylene	95040450
For large volume sample[2]: KingFisher™ 24 deep-well plate	95040470
96-well standard plates (for use with KingFisher™ Flex and KingFisher™ Apex only; tip comb placement and eluate storage):	
KingFisher™ 96 KF microplate	97002540
Tip comb, compatible with the magnetic particle processor used:	
KingFisher™ 12-tip comb, for 96 deep-well plate (for Duo Prime only)	97003500
KingFisher™ Duo Prime 6-tip comb and 24 deep-well plate (12 pieces of 24 deep-well plates, each including 4 tip combs) (for Duo Prime only)	97003510
KingFisher™ 96 tip comb for deep-well magnets, 10×10 pcs/box (for Flex and Presto)	97002534
KingFisher™ 24 deep-well tip comb and plate (for Flex and Presto)	97002610
Elution strip (for use with KingFisher™ Duo Prime only; elution step):	
KingFisher™ elution strip for 12 pin magnet (for Duo Prime only)	97003520
KingFisher™ elution strip cap for 12 pin magnet (for Duo Prime only)	97003540

(continued)

Item	Source
Equipment	
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Materials	
MicroAmp™ Clear Adhesive Film	4306311
Conical Tubes (15 mL)	AM12500
Conical Tubes (50 mL)	AM12501
Reagent reservoirs	MLS
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	AM12450
Nonstick, RNase-Free Microfuge Tubes, 2.0 mL	AM12475
Reagents	
Ethanol, 100% (molecular biology grade)	MLS
Nuclease-free water	AM9932
PBS (1X), pH 7.4	10010001
TE Buffer (0.1 mM EDTA)	12090015

[1] Standard volume sample is 200–400 µL.
[2] Large volume sample is 500 µL–2 mL.

Materials required for the OpenArray™ Plate workflow

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 Real-Time PCR Instrument with OpenArray™ block (QuantStudio™ 12K Flex OpenArray™ AccuFill™ System)	4471090
Centrifuge, capable of spinning sample plates at 1,000 × *g*	MLS
Plates and accessories	
OpenArray™ 384-well Sample Plates, black	4482221
Adhesive PCR Plate Foils	AB0626

(continued)

Item	Source
OpenArray™ AccuFill™ System Tips	4458107
QuantStudio™ 12K Flex OpenArray™ Accessories Kit[2]	4469576
Forceps	MLS
Reagents	
Genomic DNA	MLS
OpenArray™ Plates with TaqMan™ Assays	Custom ordered[3]
TaqMan™ OpenArray™ Real-Time PCR Master Mix	4462164
Ethanol, 200-proof or 80%	MLS

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

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

[3] See "Custom TrueMark™ OpenArray™ Plate formats" on page 11.

Materials required for data analysis

Item	Source
QuantStudio™ 12K Flex Software[1]	Included with QuantStudio™ 12K Flex Real–Time PCR System

[1] QuantStudio™ Design and Analysis Software v2.7 is also compatible with this workflow. For more information, contact Support.

Optional controls

Control	Purpose	How to use	Cat. No.
TrueMark™ Xeno Control, Kanamycin Resistance	Exogenous process control for DNA recovery and PCR	Nucleic acid isolation: Add to samples along with the Binding/Bead Mix	A50384
TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*)	Lyophilized organism control for nucleic acid extraction and purification	Nucleic acid isolation: Stand-alone sample, or add to samples after Enzyme Mix	A39180
TrueMark™ Wound+ABR Amplification Control	DNA plasmid control for real-time PCR	Real-time PCR: Stand-alone sample added directly to the plate	A50377

TrueMark™ Xeno Control, Kanamycin Resistance

TrueMark™ Xeno Control, Kanamycin Resistance is an exogenous process control for nucleic acid isolation and DNA recovery and PCR. The control is used with the proprietary TaqMan™ assay for Xeno™ sequences, which is included in the TrueMark™ OpenArray™ RT PCR Custom Format, Study Name "Wound+ABR Expanded Panel".

TrueMark™ Xeno Control, Kanamycin Resistance is supplied at a concentration of 2×10^5 copies/µL. During nucleic acid isolation, 10 µL of the 4-fold diluted control (50,000 copies/µL) can be added to each test sample along with the nucleic acid binding reagents (Binding Solution).

When carried through the wound microbiota and antibiotic resistance gene workflow, the control is used to monitor nucleic acid recovery and PCR. The control can be used to identify sample-specific amplification inhibition, which reduces the likelihood of false negatives and provides confidence that results are accurate. It is recommended that the control be added to each sample during nucleic acid isolation.

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

TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*) (Cat. No. A39180), serves as a process control for cell lysis and nucleic acid recovery. The control is used with the proprietary TaqMan™ Assay for *Bacillus atrophaeus* sequences.

Aliquot 10 µL of reconstituted *B. atrophaeus* (5×10^6 copies/µL) per tube, then freeze each tube at -80°C. Further dilute the reconstituted *B.atrophaeus* 40-fold with PBS (1X), pH 7.4 to a working concentration of 1.25×10^5 copies/µL for use during nucleic acid isolation.

Like other gram-positive bacteria, *Bacillus atrophaeus* has thick cell walls that can be difficult to lyse. This characteristic makes gram-positive bacteria an ideal control to monitor the efficiency of cell lysis and subsequent nucleic acid recovery.

TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*) is supplied lyophilized with a quantity of 1×10^9 copies/vial, and is reconstituted in 200 µL of 1X PBS (1X), pH 7.4 to a final concentration 5×10^6 copies/µL. During nucleic acid isolation, 10 µL of the 40-fold diluted control (1.25×10^5) is processed as a stand-alone sample in a background of Universal Transport Media (UTM). The control can be added to the negative extraction control as well as one or more test samples at the start of the extraction process. The control is carried through the remainder of the workflow with the test samples. It is recommended that at least one stand-alone control sample is run per extraction plate.

TrueMark™ Wound+ABR Amplification Control

TrueMark™ Wound+ABR Amplification Control contains a linearized multi-target plasmid with target sequences for each available wound microbiota and antibiotic resistance gene profiling assay. The plasmid also contains target sequences for the TrueMark™ Xeno Control, Kanamycin Resistance and the TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*). It can be included in wound microbiota and antibiotic resistance gene profiling experiments as a positive control for panel-specific amplification.

TrueMark™ Wound+ABR Amplification Control is supplied at a concentration of 1×10^5 copies/µL. To store, aliquot 10 µL of TrueMark™ Wound+ABR Amplification Control (1×10^5 copies/µL) per tube, then freeze each tube at -80°C.

For use in real-time PCR, dilute the TrueMark™ Wound+ABR Amplification Control 10-fold with TE Buffer (0.1 mM EDTA) to a working concentration of 1×10^4 copies/µL. During real-time PCR, 2.5 µL of the 1×10^4 copies/µL control is used as a stand-alone sample in one well of the TrueMark™ Wound+ABR Amplification Control. The control can be used to verify assay performance and help with troubleshooting.

Workflow

TrueMark™ OpenArray™ RT PCR Custom Format, Study Name "Wound+ABR Expanded Panel"

Isolate nucleic acid (page 18)
Start with wound swab samples

Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0 (page 23)

Seal and run the OpenArray™ Plates (page 39)

Analyze data (page 43)

2 Isolate nucleic acid

This chapter describes nucleic acid isolation procedure using the MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit. To isolate nucleic acid using the MagMAX™ Prime Viral/Pathogen NA Isolation Kit, refer to the *MagMAX™ Prime Viral/Pathogen NA Isolation Kit and Accessories User Guide* (“Related documentation” on page 61).

For required materials, see page 12.

Procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Ensure that the Total Nucleic Acid Binding Beads remain in a homogeneous suspension while pipetting. Vortex beads before use.

Before first use of the kit

- Download the KingFisher™ Flex script **MVP_Ultra_Flex** from the MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit (Cat. No. A42356) product page, then install it on the instrument.

  See the instrument user guide for instructions to install the script.

- Prepare fresh 80% Ethanol using 100% absolute Ethanol and Nuclease-free water, sufficient for 1.5 mL per sample, plus 10% overage.

Set up the KingFisher™ Flex instrument

- Ensure that the KingFisher™ Flex instrument has the appropriate magnetic head and heat block installed.
  - 96 deep-well magnetic head
  - 96 deep-well heat block
- Ensure that the **MVP_Ultra_Flex** script is installed on the instrument.

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

Use of the TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*) is optional.

1. Remove metal fastener from vial using tweezers and place vial on ice.
2. Remove rubber stopper from vial, then add 200 µL PBS (1X), pH 7.4 to the vial.
3. Replace the rubber stopper, then vortex the tube to mix.
4. Transfer the reconstituted control to tubes in 10 µL aliquots.

   Note: Store the reconstituted control at 4°C for up to 48 hours. For long term storage, store the reconstituted control at -80°C to -20°C for up to 4 months. Mix well to resuspend before use.

5. Thaw 1 tube (10 µL) of the reconstituted control, then add 390 µL of PBS (1X), pH 7.4 (1.25×10^5 copies/µL; 40-fold dilution).

   Note: More than 1 tube can be thawed and diluted 40-fold according to the number of samples to be extracted.

6. Add 10 µL of the 1.25×10^5 copies/µL control to each sample during nucleic acid isolation. Alternatively, TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*) can be used as a stand-alone sample.

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	100 µL
Standard[2]	6	Tip Comb	96DW Tip Comb	—

[1] KingFisher™ 96 Deep-Well Plate
[2] KingFisher™ 96 KF microplate

Set up Sample Plate, then start processing

(*Optional*) Reconstitute TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*) before use in step 3 (see "(Optional) Reconstitute TaqMan™ Universal Extraction Control Organism (B. atrophaeus)" on page 19).

1. Swirl the bottle of Enzyme Mix, then place on ice.
2. Add 50 µL of Enzyme Mix to each well in a KingFisher™ 96 Deep-Well Plate (Sample Plate).
3. Add samples and controls to the wells containing Enzyme Mix.

Sample or control	Instructions
Sample	Add 200–400 µL of sample to a well.
Negative Extraction Control (NEC)	Add 200–400 µL of Universal Transport Media to a well.
(*Optional*) TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*)	• Add 10 µL of 40-fold diluted extraction control to each sample-containing well. To create a stand-alone control well, add 10 µL of diluted extraction control to 200 µL of transport media. *or* • Add 10 µL of 40-fold diluted reconstituted control to one or more sample wells.

4. On the KingFisher™ Flex instrument, select the **MVP_Ultra_Flex** script, then press **Start**.
5. Follow the instrument prompts to load sample and processing plates, then press **Start**.

Proceed immediately to the next step.

Continue processing to bind, wash, and elute the nucleic acid

1. During the enzyme treatment incubation on the instrument, prepare the Binding/Bead Mix.
   a. Vortex the tube of Total 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
Total 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. *(Optional)* Dilute TrueMark™ Xeno Control, Kanamycin Resistance (2×10^5 copies/µL) 4-fold to a final concentration of 50,000 copies/µL with TE Buffer (0.1 mM EDTA). For a full 96-well plate total of 1,200 µL, dilute 300 µL of TrueMark™ Xeno Control, Kanamycin Resistance in 900 µL of TE Buffer (0.1 mM EDTA).

   Note: Total volume of 4-fold diluted TrueMark™ Xeno Control, Kanamycin Resistance can be calculated according to the number of samples tested. Each sample requires 10 µL of 4-fold diluted TrueMark™ Xeno Control, Kanamycin Resistance.
4. When prompted by the instrument (approximately 20 minutes after the start of the script), remove the Sample Plate from the instrument.
5. 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.
6. Gently invert the Binding/Bead Mix 5 times to mix, then use a manual pipet (single or multi-channel) to dispense 550 µL of Binding/Bead Mix 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.
7. *(Optional)* Add 10 µL of 4-fold diluted TrueMark™ Xeno Control, Kanamycin Resistance to each sample and control well in the Sample Plate.

   IMPORTANT! The TrueMark™ Xeno Control, Kanamycin Resistance must be added *after* the Binding/Bead Mix.

8. Return Sample Plate to the instrument, then press **Start** to resume the script.

9. When processing is complete (~30 minutes after adding Binding/Bead Mix), remove Elution Plate from instrument.
   The purified nucleic acid is in Elution Plate.

10. Transfer the nucleic acid samples to a 96-well storage plate or seal Elution Plate.

Store nucleic acid samples on ice for immediate use or at –20°C for longer-term storage.

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

For required materials, see “Materials required for the OpenArray™ Plate workflow” on page 14.

This chapter contains brief procedures. For detailed procedures, see the following documentation“Related documentation” on page 61.

Workflow

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

Download TPF files (page 24)

Set up the PCR reactions in an OpenArray™ 384-well Sample Plate (OpenArray™ AccuFill™ Software v2.0) (page 25)

Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0 (using an EDT) (page 26)

OR

Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0 (using a loaded TPF) (page 32)

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.
   - **TrueMark OpenArray Custom**
   - **TrueMark OpenArray Inventoried**
3. Enter the following information.

Product	Information
TrueMark 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.
TrueMark 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. For more information about setting the preferences, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* ("Related documentation" on page 61).

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. *(Optional)* Dilute the TrueMark™ Wound+ABR Amplification Control stock (1×10^5 copies/µL) 10-fold with TE Buffer (0.1 mM EDTA) to a working concentration of 1×10^4 copies/µL.

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

   (Optional) Use 2.5 µL of the 10-fold diluted TrueMark™ Wound+ABR Amplification Control (1×10^4 copies/µL) as a positive amplification control sample.

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

5. Seal the OpenArray™ 384-well Sample Plate with an aluminum foil seal by pressing hard with a delicate task wiper (e.g. Kimwipes™ Delicate Task Wipers), remove the foil flap, then mark the edges of the filled 4 × 12 area with a pen.

6. Vortex the sealed plate for 15–30 seconds to mix.

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

8. 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* (“Related documentation” on page 61).

Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0 (using an EDT)

Create an array-specific template (EDT) file

We recommend that you create a new EDT file for each new lot of OpenArray™ plates.

1. In the **Home** screen of the QuantStudio™ 12K Flex Software, in the **Experiment** pane, click **Create From Template**.

   An **Open** window appears.

2. In the **Look in** field, select **OpenArray**, click the appropriate EDT file, then click **Open**.

   A new experiment is created using the setup information from the template.

   Note: For questions about the appropriate EDT, contact Technical Support.

3. From the open experiment, click the **Targets** tab on the left pane, then click **Import** ▸ **Import Plate Setup**.

4. Click **Browse** to select the TPF plate file for the desired panel, then click **Select**.

5. Click **Start Import** ▸ **Yes** to confirm the import.

6. Click **Save As Template** to save the array-specific EDT file. The default file path for OpenArray™ EDTs is `C:\Program Files (x86)\Applied Biosystems\QuantStudio 12K Flex Software\templates`.

Map and export plate files

Configure the experiment design to map the plates

A sample plate file (CSV file) is *not* required.

Navigate to the **Map Plates** screen.

1. In the **Configure design** pane, in the **Experiment type** section, select **Gene Expression**.

2. In the **Plate format** section, select a value.

3. In the **Sample input type** section, select the type of plate.

   - **96-well**
   - **384-well**

4. In the **Pipettor** section, select a type of pipette.

   - **Fixed**
   - **Adjustable**

5. Click **Next**.

The **Map plates** pane is displayed.

- If **96-well** was selected in step 3, proceed to "Map a 384-well plate from 96-well plates" on page 27.
- If **384-well** was selected in step 3, proceed to "Set up a 384-well sample plate" on page 27.

Map a 384-well plate from 96-well plates

1. In the **Map Plates** pane, add the sample name in the 96-well plate.

   Click and drag to copy and paste sample names across a row or down a column.

   Note: The sample names are copied. They are not automatically filled. For example, if **Sample 1** is the first sample name, all of the wells that are filled by the click and drag feature are **Sample 1**. They are not named sequentially, for example, **Sample 1**, **Sample 2**, **Sample 3**.

2. *(Optional)* In the 384-well plate, click **Swap**.

   This moves the samples from the top half of the 384-well plate to the bottom half of the 384-well plate.

   Note: The **Swap** button is available only if certain experiment types and plate formats are selected. It is displayed when half of the 384-well plate is used.

3. *(Optional)* Click **Import** to import a sample file (CSV format).

   A sample file can be imported from the **Map plates** pane if it was not imported from the **Configure design** pane.

   Note: If a sample plate file is imported from the **Map plates** pane, the information overwrites any information from files that were imported from the **Configure design** pane.

Proceed to "Export plate files" on page 28.

Set up a 384-well sample plate

If the 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.

The plates are color-coded for the position on the OpenArray™ Plate based on how the experiment is configured (see "Configure the experiment design to map the plates" on page 26).

1. In the **Map Plates** pane, add the sample name to the 96-well plate.

   Click and drag to copy and paste sample names across a row or down a column.

   Note: The sample names are copied. They are not automatically filled. For example, if **Sample 1** is the first sample name, all of the wells that are filled by the click and drag feature are **Sample 1**. They are not named sequentially, for example, **Sample 1**, **Sample 2**, **Sample 3**.

2. *(Optional)* Click **Import** to import a sample plate file (CSV format).

   A sample plate file can be imported from the **Map plates** pane if it was not imported from the **Configure design** pane.

   Note: If a sample plate file is imported from the **Map plates** pane, the information overwrites any information from files that were imported from the **Configure design** pane.

3. *(Optional)* Click **Rotate data**.

   The 384-well plate can be placed inside the OpenArray™ AccuFill™ Instrument in only one orientation. If the samples were added to the physical 384-well plate in the incorrect orientation, the physical 384-well plate cannot be rotated when it is placed in the instrument for the sample plate file to match. The option to rotate the samples in the sample plate file allows the 384-well plate to be used in the correct orientation for the instrument with a matching sample plate file.

   The **Rotate data** feature is available only in the **Map Plates** screen if a 384-well sample plate file was imported. It is not accessible in the full run workflow.

Proceed to "Export plate files" on page 28.

Export plate files

1. In the **Map plates** pane, click **Export**.
   The **Select Plates to Export** dialog box is displayed.
2. Select **CSV file** as the file type to export.
3. Select the **OpenArray Plate (for QuantStudio)** as the plate file to be exported
4. Enter a prefix for the exported file name in the **File Name Prefix** field.
5. Click **OK**.

Integrate sample names before the run

Note: You can choose to integrate samples names *before* or *after* the run.

1. In the **Home** screen of the QuantStudio™ 12K Flex Software, in the **Experiment** pane, click **Create from template**.
   a. Navigate to the folder where you saved your array-specific EDT.
   b. Select the desired EDT file, then click **Open**.
2. In the **Setup** pane, click the **Samples** tab, then click **Import**.
   a. Select the OpenArray™ Plate sample information CSV generated from the Map Plates.
   b. Click **Select File**.
3. In the **Setup** pane, click the **Experimental Properties**.
   a. In the **Experiment Name** field, enter in the experiment name.

b. In the **Barcode** field, enter in the serial number of the plate you are running.

4. At the top of the screen, click **Save** ▸ **Save As** to save the file in EDS format.

5. Click **Save**.

Set up and start the run with the quick run workflow

Before you begin—quick run workflow

- Prepare samples (DNA and PCR reaction mix) in a 384-well plate, as described in "Set up the PCR reactions in an OpenArray™ 384-well Sample Plate (OpenArray™ AccuFill™ Software v2.0)" on page 25. Distribute the samples according to the layout determined in the **Map Plates** workflow.
- 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™ Plate in the plate holders.
- Clear the instrument deck, empty and replace the waste bin, and close the instrument door.
- Prepare the QuantStudio™ 12K Flex OpenArray™ Accessories Kit materials that are required to seal the OpenArray™ Plate.

IMPORTANT! OpenArray™ Plates must be sealed immediately after loading. For more information, see the user guide or application guide for your assay.

Configure the run without sample plate information

A sample plate file (CSV file) is *not* required by the OpenArray™ AccuFill™ Software v2.0.

Navigate to the **Quick Run** screen. Ensure that the **Load without sample plate information** radio button is selected.

1. *(Optional)* In the **Quick Run** screen, in the **Sample plate - optional** field, enter information to identify the sample plate.
   - Enter the information about the sample plate.
   - Use a barcode scanner to scan the sample plate or manually enter the barcode text string.
2. Select the number of samples per subarray.
   - **One**
   - **Two**
   - **Three**

3. In the **OpenArray Plate name and position** field, enter information to identify the OpenArray™ Plate.

   We recommend using the serial number of the OpenArray™ Plate as the identifying information.

   The name and position are recorded in the loading history log.

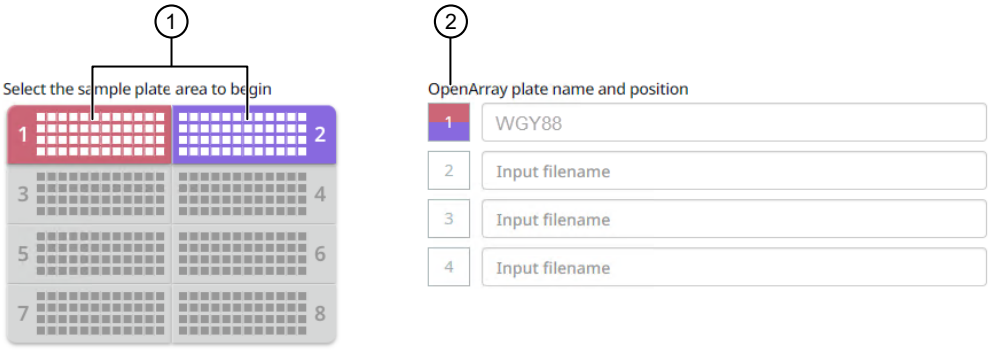
4. Click a section of the sample plate to change the corresponding OpenArray™ Plate.

   Note: The first section of the sample plate is selected. Subsequent sections are selected automatically.

   During the run, the instrument fills the OpenArray™ Plate with the samples from the corresponding section of the sample plate.

   The OpenArray™ Plate position displays the color that corresponds to the section of the sample plate.

   If there is more than one sample in a subarray, the position box displays all the colors associated with the corresponding sample.



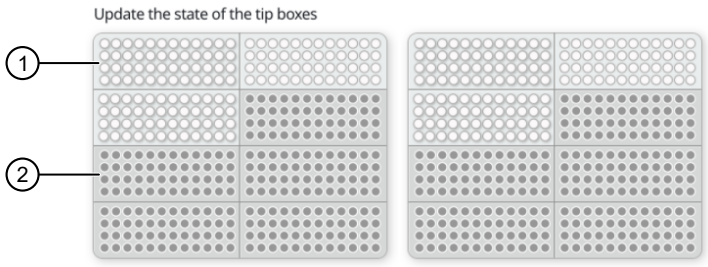
① Sample plate section
② Corresponding OpenArray™ Plate

Verify the run setup and start the run

1. Click each tip box so that the status on the **Verify the run setup and start the run** section 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 is full
② Section of the tip box is empty

2. Select the first section of the sample plate 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.

3. 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.

   The number of sections that the foil is removed from depends on how the run was set up. For example, if the run was set up without sample information and for two samples per subarray, the foil is removed from two sections at a time.

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

4. Close the instrument door.

5. 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.

IMPORTANT! Each OpenArray™ Plate must be prepared for PCR immediately after it is filled.

Remove the OpenArray™ Plate 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 within 30 seconds, 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 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 loads the next OpenArray™ Plate.

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

   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.

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

Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0 (using a loaded TPF)

Before you begin—full run workflow

- Prepare samples in a 384-well plate, as described in “Set up the PCR reactions in an OpenArray™ 384-well Sample Plate (OpenArray™ AccuFill™ Software v2.0)” on page 25.
- 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.

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.

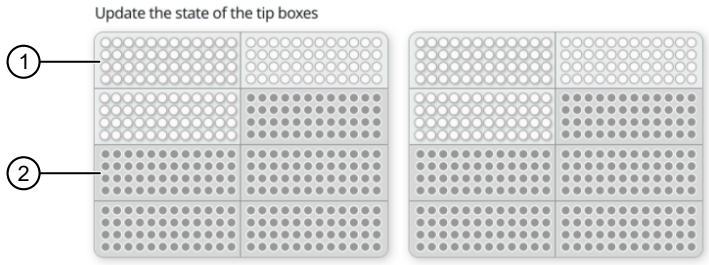
If the sample plate file was not imported, the samples must be added manually.

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

Verify the run setup and start the run

1. Click each tip box section so that the status on the **Verify and start run** pane matches the physical tip box in the instrument.

   We recommend starting the run with full tip boxes. The instrument does not start the run if there are not enough tips on the deck.



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

2. *(Optional)* Click **Auto-fill tip boxes**.

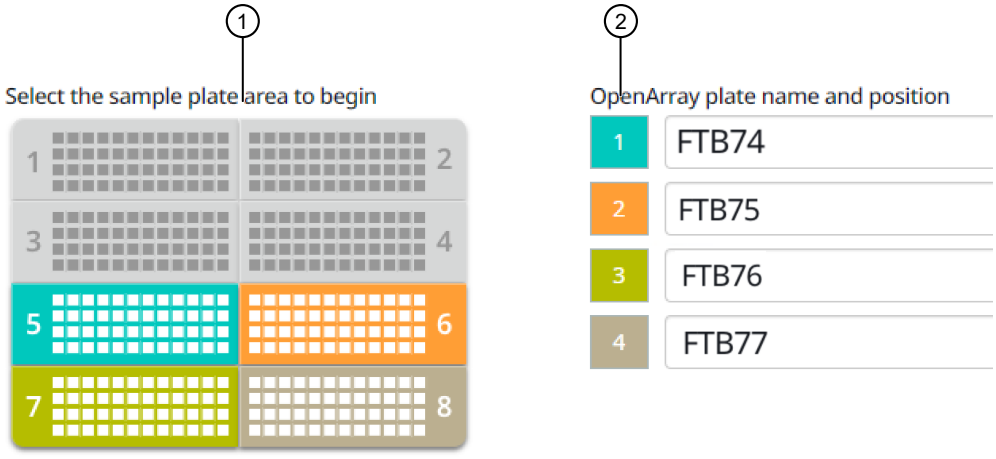
   The status of all sections of the tips boxes is set to full.

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

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

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

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



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

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

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

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

5. Close the instrument door.

6. Click **Start Run**.

   The run does not begin under any of these conditions.

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

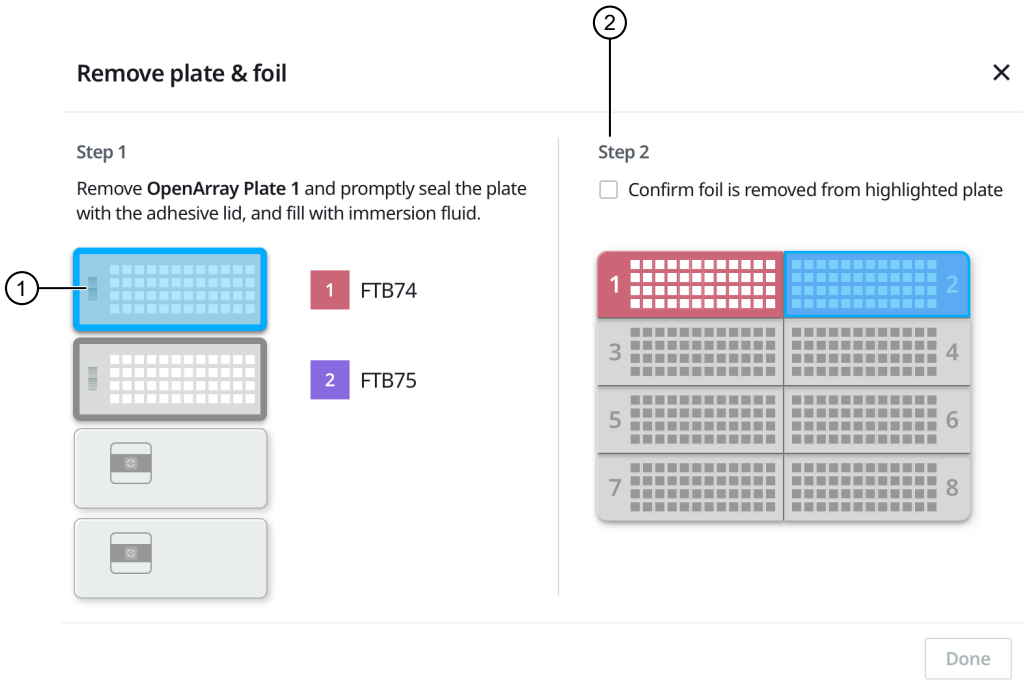


Figure 1 Remove plate and foil dialog box.

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).

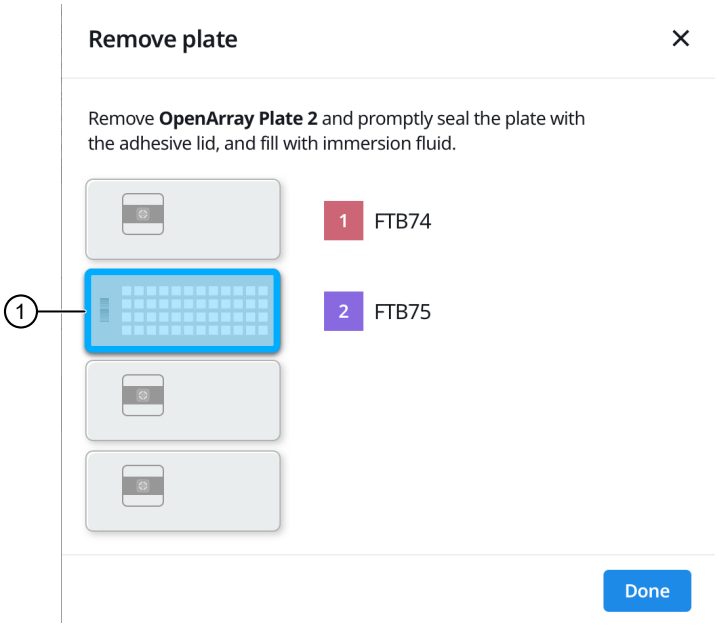


Figure 2 Remove plate dialog box

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 door is open

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

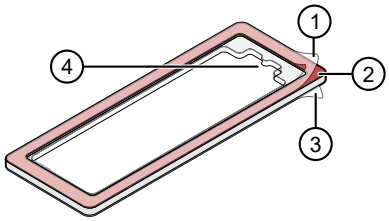


Figure 3 OpenArray™ Lid

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. Seat the lid on the OpenArray™ Plate with the lid adhesive against the plate and the notched end aligned with the serial number on the OpenArray™ Plate.

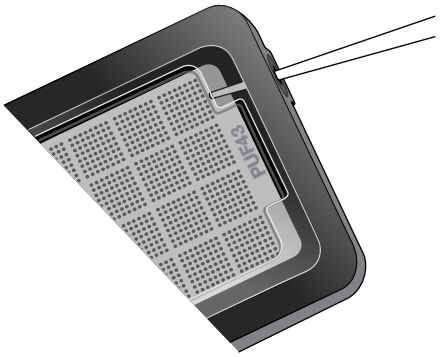
4. Engage the press mechanism until the green flashing light changes to a steady green light (approximately 20 seconds).

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, ensuring that the tip is in the front of the array, then carefully inject OpenArray™ Immersion Fluid until the case is filled.

Note:

- Minimize creation of air bubbles when you dispense the fluid.
- Leave a bubble at the fill point to prevent fluid leaks during the instrument run. The diameter of the bubble should be 4–5 mm.



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 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**. For additional information, see Appendix A, “Troubleshooting”.

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

   IMPORTANT! Create a new folder for images each time. Exporting a second run to the same folder overwrites the images.
2. Click **Export QC Images** at the bottom of the screen.
3. View the following ROX™ image to check for loading quality issues:
   - `POST-READ_CHANNEL_4.tiff`

4. Check the following spotfind 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 spotfind 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 spotfind images:
   - `STAGE2_CYCLE1_CHANNEL_1.tiff`
   - `STAGE2_CYCLE40_CHANNEL_1.tiff`

7. Note any abnormalities found, as well as all other potentially relevant information related to the setup of the run.

5 Analyze data

Select analysis software

Software	Analysis option	
	C_{rt}	QC metrics
QuantStudio™ 12K Flex Software[1]	✓	✓

[1] QuantStudio™ Design and Analysis Software v2.7 is also compatible with this workflow. For more information, contact Support.

Export data (if necessary)

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

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

Review results

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

1. In QuantStudio™ 12K Flex Software, select **Analysis Settings ▸ C_t Settings ▸ Algorithm Settings ▸ Relative Threshold**.

   The relative C_{rt} method is recommended for dried-down assays. Dried-down assays can reconstitute at different rates, causing a dip in the early cycles of the baseline. C_{rt} can correct for a variable baseline.

2. Review amplification curves (in log or linear view), C_{rt} values, and amplification curve QC metrics (Amp Score and C_q Confidence) for each reaction.

QC metric	Description
Amp Score	A value to indicate the quality of the amplification curve.
C_q Confidence	A value to reflect the reliability of the derived C_q.

3. *(Optional)* Filter data in the order indicated in the following table. Some antibiotic resistance targets require lower Amp Score and C_q Confidence cut-off values. For these individual targets, use the recommended values listed in "Recommended Amp Score and Cq Confidence thresholds for individual antibiotic resistance targets" on page 45.

Parameter to examine	Consider filtering out sample data using the following cut-off values
C_{rt}	C_{rt} >30
Amp Score	Amp Score <1.2
C_q Confidence	C_q Conf <0.7

Note:

- We encourage testing and establishing your own C_{rt} cut-off value and amplification curve QC metrics for each assay to achieve high sensitivity and specificity.
- Through-holes with unexpected C_{rt} values can also be identified by reviewing the Amplification Plot (see "Troubleshoot unexpected Crt values" on page 49).

4. Note replicates with mean C_{rt} ≤25 and a standard deviation >0.5. The data from these through-holes might require further review.

5. Ensure that at least half of the replicates amplified adequately and pass your review specifications.

6. Use your preferred method to analyze the data.

Recommended Amp Score and C_q Confidence thresholds for individual antibiotic resistance targets

Table 6 Recommended Amp Score and C_q Confidence thresholds for antibiotic resistance targets, OpenArray™ Plates

Antibiotic	Assay ID	Amp Score threshold	C_q Confidence threshold
Extended-spectrum beta-lactamases 2	Ba04646134_s1	1.1	0.5
Lincosamide, Macrolide, Streptogramin	Ba07319992_s1	1.2	0.5
Nitromidazole	Ba07922657_po	1.2	0.5
Sulfonamide	Ba00005027_po	1.2	0.5
Tetracycline 2	Ba07922656_po	1.2	0.5

Considerations for data analysis

Table 7 Species-specific assays that are also covered by pooled or broad coverage assays

Species-specific assay	Considerations for data analysis
Candida pools	The *Candida* pool 1 detects *C. albicans*, *C. parapsilosis* and *C. tropicalis*. The assay generates C_q values if any of the above *Candida* is present. The *Candida* pool 2 detects *C. glabrata* and *C. krusei*. The assay generates C_q values if any of the above *Candida* is present.
Citrobacter pool	The *Citrobacter* pool assay detects *C. braakii*, *C.freundii*, and *C.koseri*. The assay generates C_{rt} values if any of the above *Citrobacter* is present.
Clostridium pool	The *Clostridium* pool assay detects *C. histolyticum*, *C. novyi A,B*, *C. septicum* and *C. sordellii*. The assay generates C_{rt} values if any of the above *Clostridium* is present.
Klebsiella pool	The *Klebsiella* pool assay detects *K.pneumoniae* and *K.Oxytoca*. The assay generates C_{rt} values if any of the above *Klebsiella* is present.
Peptoniphilus pool	The *Peptoniphilus* pool assay detects *P. asaccharolyticus*, *P.harei*, and *P.Ivorii*. The assay generates C_{rt} values if any of the above *Peptoniphilus* is present.
Proteus pool	The *Proteus* pool assay detects *P.mirabilis* and *P.vulgaris*. The assay generates C_{rt} values if any of the above *Proteus* is present.
Staphylococcus pool	The *Staphylococcus* pool assay detects *S. epidermidis* and *S. haemolyticus*. The assay generates C_{rt} values if any of the above *Staphylococcus* is present.
Streptococcus pool	The *Streptococcus* pool assay detects *S.agalactiae*, and *S.dysgalactiae*. The assay generates C_{rt} values if any of the above *Streptococcus* is present.

Table 8 Antibiotic resistance gene-specific assays that are also covered by pool or broad coverage assays

Assay	Considerations for data analysis
AmpC beta-lactamase 1 pool	The AmpC beta-lactamase 1 pool assay detects blaACC and blaFOX genes. The assay generates C_q values if any of these genes is present.
AmpC beta-lactamase 2 pool	The AmpC beta-lactamase 2 pool assay detects blaACT and blaACT/blaMIR genes. The assay generates C_q values if any of these genes is present.
AmpC beta-lactamase 3 pool	The AmpC beta-lactamase 3 pool assay detects blaDHA, blaCMY/blaLAT, and blaMOX/blaCMY genes. The assay generates C_q values if any of these genes is present.
Carbapenemase 1 pool	The Carbapenemase 1 pool assay detects multiple blaIMP alleles. The assay generates C_q values if any of these alleles is present.
Carbapenemase 2 pool	The Carbapenemase 2 pool assay detects blaOXA-51, blaOXA-23, blaOXA-2, and blaOXA-1 genes. The assay generates C_q values if any of these genes is present.
Carbapenemase 3 pool	The Carbapenemase 3 pool assay detects blaOXA-48, blaKPC, blaVIM, and blaNDM genes. The assay generates C_q values if any of these genes is present.
Extended-spectrum beta-lactamases 1 pool	The Extended-spectrum beta-lactamases 1 pool assay detects numerous blaCTX-M alleles. The assay generates C_q values if any of these alleles is present.
Extended-spectrum beta-lactamases 3 pool	The Extended-spectrum beta-lactamases 3 pool assay detects blaGES, blaPER, and blaVEB genes. The assay generates C_q values if any of these genes is present.
Macrolides 2 pool	The Macrolides 2 pool assay detects mef(A), ere(B), and mph(A) genes. The assay generates C_q values if any of these genes is present.
Macrolides 3 pool	The Macrolides 3 pool assay detects erm(A), erm(B), and erm(C) genes. The assay generates C_q values if any of these genes is present.
Methicillin pool	The Methicillin pool assay detects mecA and mecC genes. The assay generates C_q values if any of these genes is present.
Nitromidazole pool	The Nitromidazole pool assay detects nimB, nimD, nimJ, and nimE genes. The assay generates C_q values if any of these genes is present.
Quinolone 1 pool	The Quinolone 1 pool assay detects numerous qnrB alleles. The assay generates C_q values if any of these alleles is present.
Quinolone 2 pool	The Quinolone 2 pool assay detects qnrA and qnrS genes. The assay generates C_q values if any of these genes is present.
Sulfonamide pool	The Sulfonamide pool assay detects sul1 and sul2 genes. The assay generates C_q values if any of these genes is present.
Tetracycline 2 pool	The Tetracycline 2 pool assay detects tet(A), tet(B), and tet(S) genes. The assay generates C_q values if any of these genes is present.
Trimethoprim 1 pool	The Trimethoprim 1 pool assay detects dfrA1, dfrA12, dfrA5, and dfrA17 genes. The assay generates C_q values if any of these genes is present.

Table 8 Antibiotic resistance gene-specific assays that are also covered by pool or broad coverage assays *(continued)*

Assay	Considerations for data analysis
Trimethoprim 2 pool	The Trimethoprim 2 pool assay detects dfrA14, dfrB1, dfrB5, and dfrG genes. The assay generates C_q values if any of these genes is present.
Vancomycin pool	The Vancomycin pool assay detects vanA and vanB genes. The assay generates C_q values if any of these genes is present.

Approximate C_{rt} range for controls

If used as recommended in this guide, the approximate C_{rt} range for control assays are as follows.

Control	Approximate C_{rt} range
TrueMark™ Xeno Control, Kanamycin Resistance	≤28
TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*)	≤28
TrueMark™ Wound+ABR Amplification Control	21–24

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.

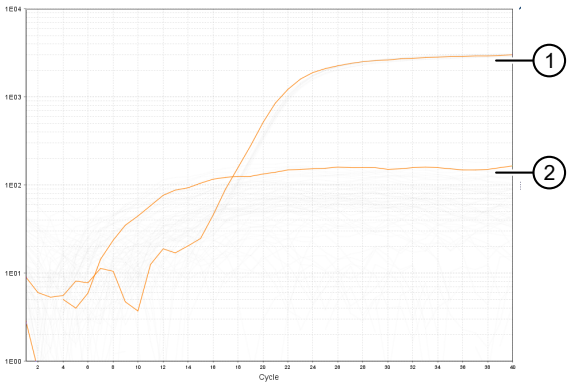
A

Troubleshooting

Troubleshooting: Nucleic Acid Isolation

Observation	Possible cause	Recommended action
Beads remain in sample after elution	Excessive amount of recovered host genomic DNA/RNA is preventing nucleic acid separation from the beads.	Increase elution volume to 100 µL.
		Reduce the input volume of starting sample to 200 µL.
Reduced extraction efficiency of TrueMark™ Xeno Control, Kanamycin Resistance	Proteinase K enzyme was either omitted from the sample or added incorrectly.	Always add Proteinase K enzyme to the sample separately and before adding the Binding/Bead Master mix.
	TrueMark™ Xeno Control, Kanamycin Resistance added at the wrong step.	Ensure that the TrueMark™ Xeno Control, Kanamycin Resistance is added to the sample well only after Binding/Bead Master mix has been added.

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, Amp Score, and C_q Confidence values.
		Consider filtering C_{rt} values from analysis.
		Compare replicates, if available.
		Dilute the samples, then repeat the experiment.
	Unexpectedly high C_{rt} values (25–28) — Sporadic amplification	Review amplification curves, Amp Score, and C_q Confidence values.
		Compare replicates, if available.
		Repeat the experiment.

Troubleshoot with cycling and imaging run images (QC images)

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

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

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

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

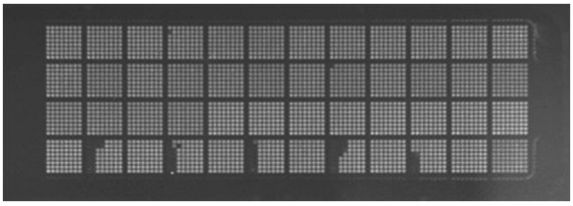
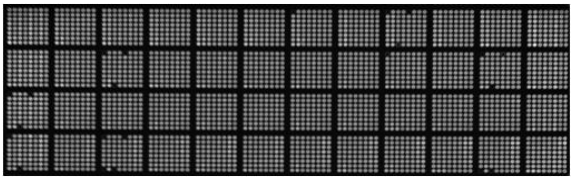
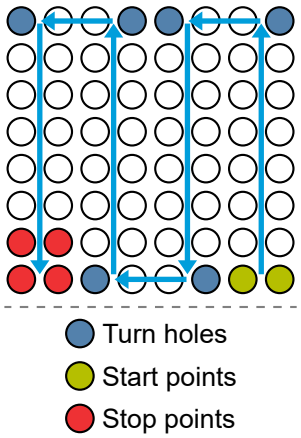
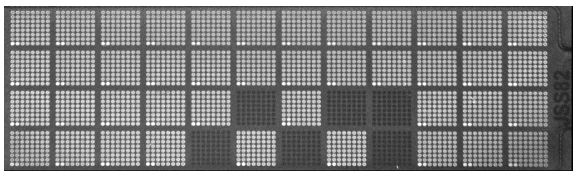
2. Use ImageJ to view the images of interest.

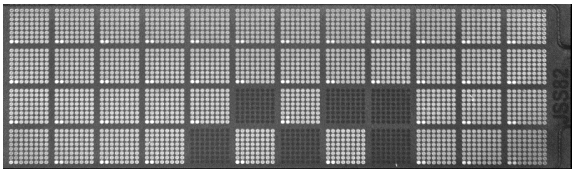
Action	Image	Image description
Confirm the identity of images within a folder	`BARCODE IMAGE.tiff`	Reflected light image of the entire OpenArray™ Plate.
Evaluate the loading quality	`PRE-READ_CHANNEL_4.tiff` `POST-READ_CHANNEL_4.tiff`	Pre- and post-ROX™ dye images.
Check for existing contamination on the case and/or heated cover	`s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff`[1]	Pre-run reflected light spotfind 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 spotfind image.
	`s02_c040_t03_p0001_m1_x2_e1_cp#_spotfind.tiff`[1]	Post-run reflected light spotfind 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.

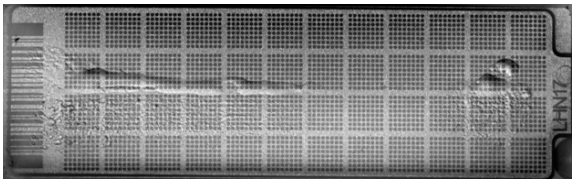
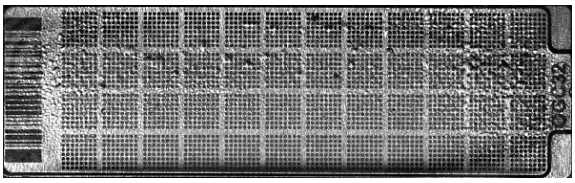
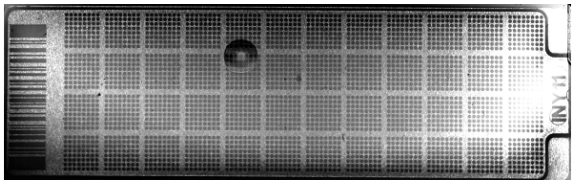
3. *(Optional)* Adjust the images for brightness and/or contrast to make image features visible.
   a. Open the image in ImageJ.
   b. Select **Image ▸ Adjust Brightness/Contrast** (or press **Carl+Shift+C**).
   c. Click **Auto** or adjust the sliders until the features of interest in the image are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

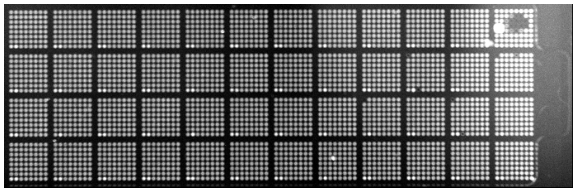
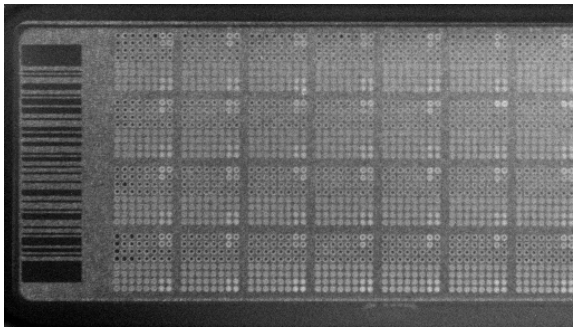
OpenArray™ AccuFill™ Instrument plate loading errors

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 was aligned too far to the left or to the right. Systematic loading problems can occur with the OpenArray™ AccuFill™ Instrument, which indicates a need for service. For example, when turn-holes are repeatedly missed across multiple subarrays, service is required. Turn-holes are where the instrument changes direction during sample loading. 	Contact your local field service engineer.
Entire subarrays are missing 	The sample/master mix was not added to particular wells in the 384-well Sample Plate.	Visually inspect the plate to ensure that the wells have sample/master mix.
	Stuck tip mandrel on the OpenArray™ AccuFill™ Instrument may need cleaning.	Contact your local field service engineer.

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

OpenArray™ Plate assembly and handling errors

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

Observation	Possible cause	Recommended action
windows and obscured the view of the through-holes. The best image in which to detect leaks is the `s02_c001_t03_p0001_m1_x2_e1_cp#_spotfind.tiff` image. This image is taken at the start of cycling, which is where most leaks occur. See "Troubleshoot with cycling and imaging run images (QC images)" on page 49.	Too large of a bubble inside the OpenArray™ Case after sealing.	Minimize the size of the bubble by tilting the OpenArray™ Case so that the fill port is at the highest point. Slowly fill the case with immersion fluid until only a small air bubble remains. Attach the screw and wipe off any excess oil that may have spilled onto the case.
	Damaged plate press, leading to uneven pressure.	Contact your field service engineer if you suspect that your plate press may be damaged.
Sample blow-out during the addition of immersion fluid 	The reactions in A12 were compromised during the addition of immersion fluid. Injecting the immersion fluid too quickly can purge the sample out of the through-holes near the fill port. Often this is caused by the user not purging the syringe slightly before use or if the syringe is not inserted into the correct side of the steel plate.	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 TaqMan™ assay pools included in the TrueMark™ OpenArray™ RT PCR Custom Format, Study Name "Wound+ABR Expanded Panel"—component assay IDs

The following table lists component assay IDs within each pool. To order the correct pool, use the pool assay IDs listed in “TaqMan™ assays for wound microbiota and antibiotic resistance gene targets” on page 7.

Table 9 Assay pools for wound microbiota targets—component assay IDs

#[1]	Pathogen	Bacterial gram strain	Assay ID
4	*Candida albicans*	N/A	Fn04646233_s1
	Candida parapsilosis		Fn04646221_s1
	Candida tropicalis		Fn04646220_s1
5	*Candida glabrata*	N/A	Fn04646240_s1
	Candida krusei		Fn04646223_s1
6	*Citrobacter braakii*	Gram-negative	Ba07922291_s1
	Citrobacter freundii	Gram-negative	Ba07286616_s1
	Citrobacter koseri	Gram-negative	Ba07921943_s1
8	*Clostridium histolyticum*	Gram-positive	Ba07922561_s1
	Clostridium novyi A,B	Gram-positive	Ba07922185_s1
	Clostridium septicum	Gram-positive	Ba07922180_s1
	Clostridium sordellii	Gram-positive	Ba07922558_s1
16	*Klebsiella oxytoca*	Gram-negative	Ba04932079_s1
	Klebsiella pneumoniae	Gram-negative	Ba04932083_s1
18	*Peptoniphilus asaccharolyticus*	Gram-positive	Ba07922560_s1
	Peptoniphilus harei	Gram-positive	Ba07922183_s1

Table 9 Assay pools for wound microbiota targets—component assay IDs *(continued)*

#[1]	Pathogen	Bacterial gram strain	Assay ID
18	*Peptoniphilus ivorii*	Gram-positive	Ba07922563_s1
20	*Proteus mirabilis*	Gram-negative	Ba04932076_s1
	Proteus vulgaris	Gram-negative	Ba04932082_s1
25	*Staphylococcus epidermidis*	Gram-positive	Ba04646141_s1
	Staphylococcus haemolyticus	Gram-positive	Ba07922176_s1
29	*Streptococcus agalactiae*	Gram-positive	Ba04646276_s1
	Streptococcus dysgalactiae	Gram-positive	Ba07921957_s1

[1] From Table 1 on page 7.

Table 10 Assay pools for antibiotic resistance targets—component assay IDs

#[1]	Antibiotic	Gene family	Assay ID
1	AmpC beta-lactamase 1	blaACC	Ba04646144_s1
		blaFOX	Ba04646126_s1
2	AmpC beta-lactamase 2	blaACT	Ba04646117_s1
		blaACT/blaMIR	Ba04646124_s1
3	AmpC beta-lactamase 3	blaCMY/blaLAT	Ba04646135_s1
		blaDHA	Ba04646120_s1
		blaMOX/blaCMY	Ba04646156_s1
5	Carbapenemase 1	blaIMP	Ba04646119_s1
			Ba04646131_s1
			Ba04646116_s1
			Ba04646158_s1
6	Carbapenemase 2	blaOXA-1	Ba04646133_s1
		blaOXA-2	Ba07922582_s1
		blaOXA-23	Ba04646139_s1
		blaOXA-51	Ba07319995_s1
7	Carbapenemase 3	blaKPC	Ba04646152_s1
		blaNDM	Ba04646121_s1
		blaOXA-48	Ba04646138_s1

Table 10 Assay pools for antibiotic resistance targets—component assay IDs *(continued)*

#[1]	Antibiotic	Gene family	Assay ID
7	Carbapenemase 3	blaVIM	Ba07922583_s1
8	Extended-spectrum beta-lactamases 1	blaCTX-M	Ba04646127_s1
			Ba04646142_s1
			Ba04646149_s1
			Ba07922581_s1
10	Extended-spectrum beta-lactamases 3	blaGES	Ba04646151_s1
		blaPER	Ba04646140_s1
		blaVEB	Ba04646153_s1
13	Macrolides 2	ere(B)	Ba07319981_s1
		mef(A)	Ba07922579_s1
		mph(A)	Ba07922580_s1
14	Macrolides 3	erm(A)	Ba04646137_s1
			Ba07922565_s1
		erm(B)	Ba04230913_s1
		erm(C)	Ba07319994_s1
15	Methilcillin	mecA	Ba04230908_s1
		mecC	Ba07319993_s1
16	Nitromidazole	nimB	Ba07319983_s1
		nimD	Ba07320001_s1
		nimE	Ba07319999_s1
		nimJ	Ba07320000_s1
17	Quinolone 1	qnrB	Ba07922571_s1
			Ba07922572_s1
			Ba07922569_s1
18	Quinolone 2	qnrA	Ba07922584_s1
		qnrS	Ba04646145_s1
19	Sulfonamide	sul1	Ba07319988_s1
		sul2	Ba07320003_s1

Table 10 Assay pools for antibiotic resistance targets—component assay IDs *(continued)*

#[1]	Antibiotic	Gene family	Assay ID
21	Tetracycline 2	tet(A)	Ba07922566_s1
		tet(B)	Ba07921939_s1
		tet(S)	Ba07319979_s1
22	Trimethoprim 1	dfrA1	Ba07319989_s1
		dfrA5	Ba07319986_s1
		dfrA12	Ba07922567_s1
		dfrA17	Ba07922575_s1
23	Trimethoprim 2	dfrA14	Ba07922574_s1
		dfrB1	Ba07922576_s1
		dfrB5	Ba07922578_s1
		dfrG	Ba07922577_s1
24	Vancomycin	vanA	Ba04646147_s1
		vanB	Ba04646150_s1

[1] From Table 2 on page 8.

C 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, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  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

D Documentation and support

Related documentation

Document	Publication Number
MagMAX™ Prime Viral/Pathogen NA Isolation Kit and Accessories User Guide	MAN0029683
OpenArray™ Sample Tracker Software Quick Reference, for OpenArray™ AccuFill™ Software v1.2	4460657
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
QuantStudio™ 12K Flex 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
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935
QuantStudio™ 12K Flex Real–Time PCR System v1.4 Maintenance and Administration Guide	4470689
QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide	MAN0018832
Thermo Scientific™ KingFisher™ Flex User Manual	MAN0019870
TaqMan™ Universal Extraction Control Organism (B. atrophaeus) Product Information Sheet	MAN0018535
TrueMark™ Xeno Control, Kanamycin Resistance Product Information Sheet	MAN0026587

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  - 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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