TrueMark™ Wound Microbiota and Antibiotic Resistance Gene Profiling Experiment—TaqMan™ Array Card format

USER GUIDE

TaqMan™ Assays for wound microbiota and antibiotic resistance gene profiling experiments in TaqMan™ Array Card format

for use with:

TaqMan™ Microbial Array Specialty Card, Study Name "Wound+ABR Expanded Panel"

Custom TaqMan™ Array Cards

MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit

QuantStudio™ 12K Flex Real–Time PCR System

QuantStudio™ 7 Flex Real-Time PCR System

Catalog Number 4398986

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

Revision history: MAN0030009 B.0 (English)

Revision	Date	Description
B.0	8 February 2024	• Updated the list of assays.
A.0	11 September 2023	New user guide for TrueMark™ Wound Microbiota and Antibiotic Resistance Gene Profiling Experiment—TaqMan™ Array Card format.

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

Contents

1 Overview

Product description

TaqMan™ Microbial Array Specialty Card, Study Name "Wound+ABR Expanded Panel" (Cat. No. 4398986) is an efficient, easy-to-use TaqMan™ Array Card for the characterization of key wound microbiota targets and antibiotic resistance gene families. The array card includes TaqMan™ assays that have been optimized for detection of bacteria, fungi, and antibiotic resistance gene targets. The array card 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 included in the TaqMan™ Microbial Array Specialty Card, Study Name "Wound+ABR Expanded Panel"” on page 6.

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 also undergo performance testing to ensure that results are accurate and reproducible with high levels sensitivity and specificity.

TaqMan™ assays included in the TaqMan™ Microbial Array Specialty Card, Study Name "Wound+ABR Expanded Panel"

The following tables list assays that are included in the TaqMan™ Microbial Array Specialty Card, Study Name "Wound+ABR Expanded Panel".

The assays can also be ordered in a Custom TaqMan™ Array Card.

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	*Clostridium perfringens*	Gram-positive	Ba07922559_s1
7	*Clostridium histolyticum*	Gram-positive	Ba07922644_po[1]
	Clostridium novyi A,B	Gram-positive	
	Clostridium septicum	Gram-positive	
	Clostridium sordellii	Gram-positive	
8	*Enterobacter cloacae*	Gram-negative	Ba04932087_s1
9	*Enterococcus faecalis*	Gram-positive	Ba04646247_s1
10	*Enterococus faecium*	Gram-positive	Ba04932086_s1
11	*Escherichia coli*	Gram-negative	Ba04646242_s1
12	*Finegoldia magna*	Gram-positive	Ba07921950_s1
13	*Klebsiella aerogenes*	Gram-negative	Ba04932080_s1
14	*Klebsiella oxytoca*	Gram-negative	Ba00005014_po[1]
	Klebsiella pneumoniae	Gram-negative	
15	*Morganella morganii*	Gram-negative	Ba04932078_s1

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

#	Pathogen	Bacterial gram strain	Assay ID
16	*Proteus mirabilis*	Gram-negative	Ba00005020_po[1]
	Proteus vulgaris	Gram-negative	
17	*Pseudomonas aeruginosa*	Gram-negative	Ba04932081_s1
18	*Serratia marcescens*	Gram-negative	Ba07921916_s1
19	*Staphylococcus aureus*	Gram-positive	Ba04646259_s1
20	*Staphylococcus lugdunensis*	Gram-positive	Ba07921980_s1
21	*Streptococcus agalactiae*	Gram-positive	Ba07922868_po[1]
	Streptococcus anginosus	Gram-positive	
	Streptococcus dysgalactiae	Gram-positive	
22	*Streptococcus pyogenes*	Gram-positive	Ba07921919_s1
23	*Providencia stuartii*	Gram-negative	Ba04932077_s1
24	*Peptoniphilus asaccharolyticus*	Gram-positive	Ba07922642_po[1]
	Peptoniphilus harei	Gram-positive	
	Peptoniphilus ivorii	Gram-positive	
25	*Peptostreptococcus anaerobius*	Gram-positive	Ba07921938_s1

[1] To order the correct pool, use assay IDs listed in this table. For component assay IDs within each pool, see Table 11 on page 43.

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]
		blaDHA	
		blaMOX/blaCMY	
4	Beta-Lactamase	blaTEM	Ba04646128_s1
5	Carbapenemase 1	blaIMP	Ba07922413_po[1]
6	Carbapenemase 2	blaOXA-1	Ba07922647_po[1]

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

#	Antibiotic	Gene family	Assay ID
6	Carbapenemase 2	blaOXA-2	Ba07922647_po[1]
		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	mef(A)	Ba07922869_po[1]
		ere(B)	
		mph(A)	
		msr(A)	
13	Macrolides 2	erm(A)	Ba07922653_po[1]
		erm(B)	
		erm(C)	
14	Methicillin	mecA	Ba07922654_po[1]
		mecC	
15	Nitromidazole	nimB	Ba07922657_po[1]
		nimD	
		nimE	
		nimJ	
16	Quinolone 1	qnrB	Ba07922658_po[1]
17	Quinolone 2	qnrA	Ba07922659_po[1]

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

#	Antibiotic	Gene family	Assay ID
17	Quinolone 2	qnrS	Ba07922659_po[1]
18	Sulfonamide	sul1	Ba00005027_po[1]
		sul2	
19	Tetracycline	tet(A)	Ba07922870_po[1]
		tet(B)	
		tet(M)	
		tet(S)	
20	Trimethoprim	dfrA1	Ba07922651_po[1]
		dfrA5	
		dfrA12	
		dfrA17	
21	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 12 on page 44.

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

TaqMan™ Array Card products and formats

TaqMan™ Microbial Array Specialty Card, Study Name "Wound+ABR Expanded Panel"

The TaqMan™ Microbial Array Specialty Card, Study Name "Wound+ABR Expanded Panel" (Cat. No. 4398986) contains pre-plated, dried down TaqMan™ assays for wound microbiota and antibiotic resistance gene profiling. For the complete list of assays included with the array card, see “TaqMan™ assays included in the TaqMan™ Microbial Array Specialty Card, Study Name "Wound+ABR Expanded Panel"” on page 6.

Contents and storage

Table 4 TaqMan™ Microbial Array Specialty Card, Study Name "Wound+ABR Expanded Panel" (Cat. No. 4398986)

Component	Amount	Storage
TaqMan™ Microbial Array Specialty Card, Study Name "Wound+ABR Expanded Panel"	10 cards[1]	2°C to 8°C

[1] Minimum order.

Custom TaqMan™ Array Card formats

Custom layouts can be configured using Assay IDs listed in “TaqMan™ assays included in the TaqMan™ Microbial Array Specialty Card, Study Name "Wound+ABR Expanded Panel"” on page 6, along with other TaqMan™ microbe detection assays.

Array format	Number of assays/replicated	Maximum number of samples
24	24/2	8
48	48/1	8

Configure and order Custom TaqMan™ Array Cards

1. Go to **https://www.thermofisher.com/microbe-detection/taqman/target-list/choose-format**.
2. For the format, select **Plates or cards**, then click **Next step**.
3. Enter the list of targets or import an existing list, then click **Submit**. When complete, click **Next step**.
4. *(Optional)* To add additional targets, click **Add another**. When complete, click **Next step**.
5. Follow the on-screen instructions to configure the assays on the plate.
6. *(Optional)* Click **Save progress** at any time to save the array configuration to your Thermo Fisher Scientific account.
7. When the plate is configured, click **Next step**, then click **Get quote**.
8. Follow the on-screen instructions to contact the **Specialty Array team**.

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 50).

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	
Proteinase K	1 mL	
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

(continued)

Item	Source
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
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

(continued)

Item	Source
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 PCR

Table 6 Materials required for real-time PCR

Item	Source
Equipment	
Microcentrifuge	MLS
Vortex mixer	MLS
Micropipettes	MLS
Tubes, plates, and other consumables	
MicroAmp™ Optical 96-Well Reaction Plate	N8010560, or equivalent; see **thermofisher.com/plastics**
MicroAmp™ Clear Adhesive Film	4306311
Aerosol-resistant barrier pipette tips	MLS
Disposable gloves	MLS
Reagents	
Nuclease-free water	AM9937, or equivalent

Table 7 Additional materials required for real-time PCR with TaqMan™ Array Cards

Item	Source
Real-time PCR instrument, one of the following; configured with the TaqMan™ Array Card block and heated cover.	
QuantStudio™ 7 Flex Real-Time PCR System	Contact your local sales office
QuantStudio™ 12K Flex Real–Time PCR System	
Equipment	
TaqMan™ Array Card Sealer	4331770

Table 7 Additional materials required for real-time PCR with TaqMan Array Cards *(continued)*

Item	Source
Centrifuge with custom buckets and card holders, one of the following: • Sorvall™ centrifuge • Heraeus™ centrifuge See the **Resources** section at **thermofisher.com/taqmanarrays** for a list of compatible centrifuges, rotors, and buckets.	Contact your local sales office
Blank balance TaqMan™ Array Cards (Included with the instrument block upgrade / installation kit)	Contact your local sales office
Reagents	
TaqPath™ BactoPure™ Microbial Detection Master Mix	A52699 (1 mL)
	A52700 (5 mL)

Materials required for data analysis

Item	Source
Applied Biosystems™ QuantStudio™ Design and Analysis Software v2.6 or later *(recommended)*	**thermofisher.com/qpcrsoftware**

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 TaqMan™ Microbial Array Specialty Card, 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, 10 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

TaqMan™ Microbial Array Specialty Card, Study Name "Wound+ABR Expanded Panel"

Isolate nucleic acid (page 17)
Start with wound swab samples

Prepare and run TaqMan™ Array Cards (page 22)

Analyze data (page 24)

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 50).

For required materials, see page 11.

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 18).

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 and run TaqMan™ Array Cards

For detailed instructions for handling TaqMan™ Array Cards, see Appendix B, "Detailed procedures for preparation of a TaqMan™ Array Card".

Download the plate file for the array card at **thermofisher.com/taqmanfiles**.

1. Allow the card to equilibrate to room temperature.
2. Gently mix the bottle of TaqPath™ BactoPure™ Microbial Detection Master Mix.
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. For each port, combine the following components.

   (*Optional*) Use 10 µL of the 10-fold diluted TrueMark™ Wound+ABR Amplification Control (1×10^4 copies/µL) plus 10 µL of nuclease-free water instead of eluted sample as a positive amplification control sample.

Component	Volume per port
Eluted sample	20 µL
TaqPath™ BactoPure™ Microbial Detection Master Mix	50 µL
Nuclease-free water	30 µL
Total volume per port	**100 µL**

5. Fill each port with 100 µL of prepared mix.
6. Centrifuge the card at 1,200 rpm (301 × *g*) for 1 minute.
7. Repeat step 6.
8. Seal the card using TaqMan™ Array Card Sealer (see "Seal the card" on page 40 for detailed instructions).
9. Load the card into the real-time PCR instrument, then set up the experiment in the instrument software.
   - Experiment type—**Array Card**
   - Experiment—**Standard curve**
   - Run type—**Fast**
   - Sample and assay assignments—Import the plate file (TXT) for the card, then assign samples.

- Run method—Change the default run method to the following settings:

Step	Stage	Cycles	Temp.	Time
Activation	1	1	95°C	10 minutes
Amplification	2	40	95°C	3 seconds
			60°C	30 seconds

10. Run the program.

4 Analyze data

Recommended analysis software

Software	Analysis option	
	Relative threshold analysis	QC metrics
QuantStudio™ Design and Analysis Software v2.6 or later	✓	✓

Review results

1. In the analysis settings of the software, select the relative threshold method.

   The relative threshold 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. Relative threshold can correct for a variable baseline.

   a. In QuantStudio™ Design and Analysis Software v2.6 or later, from the **Actions** dropdown list, select **Primary Analysis Setting**.

   b. In the **Primary Analysis Setting** dialog, in the **General** tab, select **Relative Threshold** from the **Algorithm Settings** dropdown list.

2. Review amplification curves (in log or linear view), C_q 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 25.

Note: We encourage testing and establishing your own C_q cut-off value and amplification curve QC metrics for each assay to achieve high sensitivity and specificity.

Parameter to examine	Consider filtering out sample data using the following cut-off values
C_q	C_q >35
Amp Score	Amp Score <1.2
C_q Confidence	C_q Confidence <0.7

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

Table 8 Recommended Amp Score and C_q Confidence thresholds for antibiotic resistance targets, TaqMan™ Array Card

Antibiotic	Assay ID	Amp Score threshold	C_q Confidence threshold
AmpC beta-lactamase 2	Ba07922650_po	1.2	0.6
Extended-spectrum beta-lactamases 1	Ba07922646_po	1.2	0.6
Macrolides 1	Ba07922869_po	1.2	0.6
Sulfonamide	Ba00005027_po	1.2	0.6
Trimethoprim	Ba07922651_po	1.1	0.6

Considerations for data analysis

Table 9 Species-specific assays that are also covered by pool 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.
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_q values if any of the above *Klebsiella* is present.
Proteus pool	The *Proteus* pool assay detects *P.mirabilis* and *P.vulgaris*. The assay generates C_q values if any of the above *Proteus* is present.

Table 9 Species-specific assays that are also covered by pool or broad coverage assays *(continued)*

Species-specific assay	Considerations for data analysis
Streptococcus pool	The *Streptococcus* pool assay detects *S.agalactiae*, *S.dysgalactiae*, and *S.anginosus*. The assay will generate C_q values if any of the above *Streptococcus* is present.
Peptoniphilus pool	The *Peptoniphilus* pool assay detects *P. asaccharolyticus*, *P.harei*, and *P.Ivorii*. The assay generates C_q values if any of the above *Peptoniphilus* is present.

Table 10 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 are 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 are 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 are present.
Carbapenemase 1 pool	The Carbapenemase 1 pool assay detects blaIMP gene. The assay generates C_q values if blaIMP gene 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 are 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 are present.
Extended-spectrum beta-lactamases 1 pool	The Extended-spectrum beta-lactamases 1 pool assay detects blaCTX-M gene. The assay generates C_q values if blaCTX-M gene 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 are present.
Macrolides 1 pool	The Macrolides 1 pool assay detects mef(A), ere(B), mph(A), and msr(A) genes. The assay generates C_q values if any of these genes are present.
Macrolides 2 pool	The Macrolides 2 pool assay detects erm(A), erm(B), and erm(C) genes. The assay generates C_q values if any of these genes are present.
Methicillin pool	The Methicillin pool assay detects mecA and mecC genes. The assay will generate C_q values if any of these genes are 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 are present.
Quinolone 1 pool	The Quinolone 1 pool assay detects qnrB gene. The assay generates C_q values is qnrB gene 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 are present.

Table 10 Antibiotic resistance gene-specific assays that are also covered by pool or broad coverage assays *(continued)*

Assay	Considerations for data analysis
Sulfonamide pool	The Sulfonamide pool assay detects sul1 and sul2 genes. The assay generates C_q values if any of these genes are present.
Tetracycline pool	The Tetracycline pool assay detects tet(M), tet(A), tet(B), and tet(S) genes. The assay generates C_q values if any of these genes are present.
Trimethoprim pool	The Trimethoprim pool assay detects dfrA1, dfrA12, dfrA5, and dfrA17 genes. The assay generates C_q values if any of these genes are present.
Vancomycin pool	The Vancomycin pool assay detects vanA and vanB genes. The assay generates C_q values if any of these genes are present.

Approximate C_q range for controls

If used as recommended in this guide, the approximate C_q range for control assays are as follows.

Control	Approximate C_q 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_q**, **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_q	Average of C_q
	StdDev of C_q[2]	StdDev of C_q[2]
	Count of C_q	Count of C_q

(continued)

Area of pivot table	Fields to add	
	Target-oriented view	Sample-oriented view
Values	—	**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.	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 Binding/Bead Master mix before dispensing into sample wells.

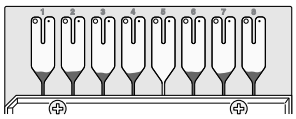
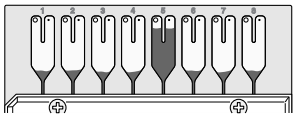
Troubleshooting: After removing the card from packaging

Observation	Possible cause	Recommended action
Water condenses on the reaction wells (optical side of the card)	The card was not at room temperature before being removed from the packaging.	Remove condensation on the reaction wells by lightly blowing room temperature pressurized nitrogen or an air blower on the wells. **IMPORTANT!** Ensure that all water condensation is removed. The optical side of the card must be free of water condensation.

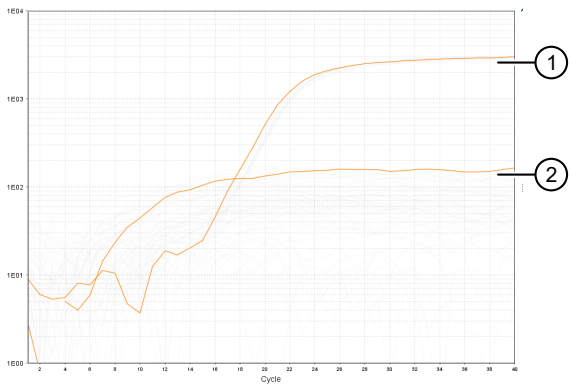
Troubleshooting: After loading PCR reaction mix into the card

Observation	Possible cause	Recommended action
Fill reservoirs have bubbles in the PCR reaction mix	When loading the card with PCR reaction mix, air was introduced into the fill reservoir.	Inspect the affected rows after centrifuging and sealing the card. Note wells that contain bubbles, then consider omitting these wells from analysis.
Fill reservoir is not full of PCR reaction mix	The PCR reaction mixture was not correctly pipetted into the fill reservoir.	Be sure to correctly pipette the entire PCR reaction mixture (100 µL) into the fill reservoir. Add more sample-specific PCR reaction mix to the fill reservoir.
PCR reaction mix leaks from the vent port into the fill reservoir	The PCR reaction mixture was not correctly pipetted into the fill reservoir.	Be sure to correctly pipette the entire PCR reaction mixture (100 µL) into the fill reservoir. Add more sample-specific PCR reaction mix to the fill reservoir.

Troubleshooting: After centrifuging the card

Observation	Possible cause	Recommended action
Fill reservoir completely empty 	Some wells were filled improperly.	Continue with running the card. However, consider omitting the wells associated with that fill reservoir.
PCR reaction mix remains in a fill reservoir 	Though rare, the fill port is blocked.	Inspect the card for blocked fill port or a pinched channel. If the fill reservoir is defective, contact Support.
	Filling is incomplete or not consistent.	Centrifuge the card again for 1 minute.
		If the filling is still incomplete after the additional centrifuge cycle, continue with running the card. However, consider omitting the wells associated with that fill reservoir.

Troubleshooting: After running the card and reviewing run results

Observation	Possible cause	Recommended action
Unexpected C_q values in the amplification plot  ① Expected C_q value (noted in most replicates) ② Unexpected C_q value (too low)	Unexpectedly low C_q values (<10) — Signal variation or noise in early PCR cycles	Review amplification curves, Amp Score, and C_q Confidence values.
		Consider filtering C_q values from analysis.
		Compare replicates, if available.
		Dilute the samples, then repeat the experiment.
	Unexpectedly high C_q values (27–30) — Sporadic amplification	Review amplification curves, Amp Score, and C_q Confidence values.
		Compare replicates, if available.
		Repeat the experiment.

Observation	Possible cause	Recommended action
No amplification in some wells	Empty wells due to improper card sealing.	When sealing the card, use a slow and steady motion to push the carriage across the TaqMan™ Array Card Sealer. **IMPORTANT!** Do not move the carriage back across the card. See "Seal the card" on page 40.
	Positive sample is not present in the wells.	No action required. Consider using one or more positive controls to confirm nucleic acid recovery.
No amplification for portions of the card	The card was misaligned in the block during the instrument run.	Inspect the card for crushed or distorted feet. If there are damaged feet, contact Support.  ① Array card feet
	Positive sample is not present in the wells.	No action required. Consider using one or more positive controls to confirm nucleic acid recovery.
No amplification within or across one or more rows	Empty wells due to improper card sealing.	When sealing the card, use a slow and steady motion to push the carriage across the TaqMan™ Array Card Sealer.
	Empty wells due to misalignment of the TaqMan™ Array Card Sealer.	If the TaqMan™ Array Card Sealer is misaligned, contact Support.
	PCR reaction mix improperly prepared.	Ensure that all reaction components were added to the PCR reaction mix.
	Positive sample is not present in the wells.	No action required. Consider using one or more positive controls to confirm nucleic acid recovery.
The baseline is variable	The dried-down assays on the card were reconstituted at different rates, causing a dip in the early cycles of the baseline.	Use the relative threshold algorithm. The relative threshold algorithm can correct for a variable baseline.

Observation	Possible cause	Recommended action
Noise in the amplification plots for portions of the card	The card was misaligned in the block during the instrument run.	Inspect the card for crushed or distorted feet. If there are damaged feet, contact Support.  1 Array card feet
A gradient signal across the card	The card was in a diagonal position during centrifugation because not all of the positions in the card holder were filled.	Repeat the assay with a new card. Ensure that all the positions in the centrifuge card holder are filled.

B

Detailed procedures for preparation of a TaqMan™ Array Card

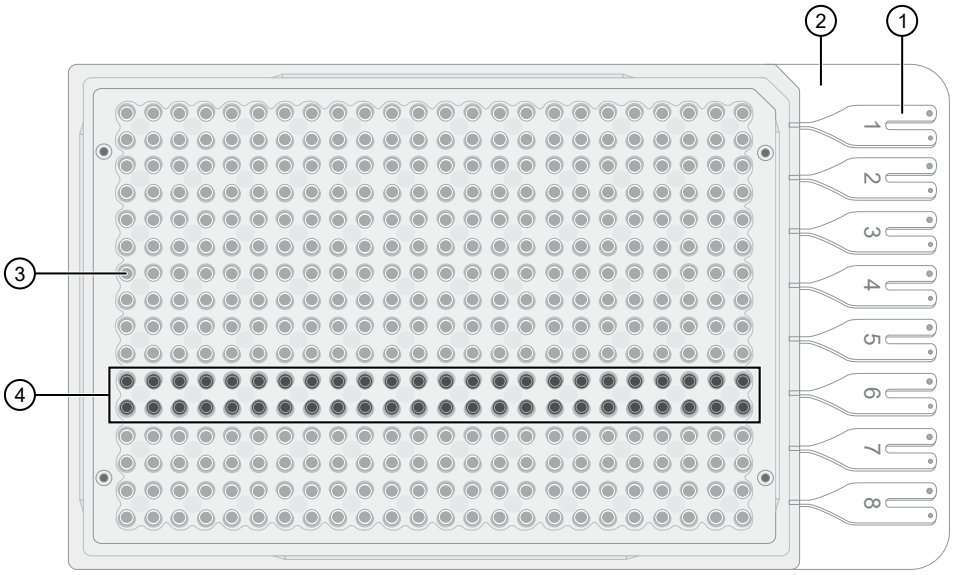
TaqMan™ Array Card overview

TaqMan™ Array Cards are 384-well microfluidic cards that are prepared with dried-down TaqMan™ Assays.

Advantages of using TaqMan™ Array Cards include:

- Small-volume design that minimizes sample and reagent consumption.
- Streamlined reaction setup that saves time and reduces labor-intensive steps.
- Access to high-throughput, 384-well format without liquid-handling robotics.
- Two-fold discrimination detection at the 99.7% confidence level.
- Standardization across multiple samples in multiple laboratories.

Each card can run 1 to 8 samples against 12 to 384 TaqMan™ Assay targets (including controls).



① **Fill reservoir**—Each reservoir is loaded with a sample-specific PCR reaction mix; the associated reaction wells fill with that sample (8 total reservoirs)

② **Fill reservoir strip**—Support strip for fill reservoirs; removed before running the card

③ **Reaction well**—Each well contains dried-down assay (384 total reaction wells)

④ **Reaction well row**—A set of reaction wells that fill with the same sample-specific PCR reaction mix (8 total rows, each row associated with a single fill reservoir)

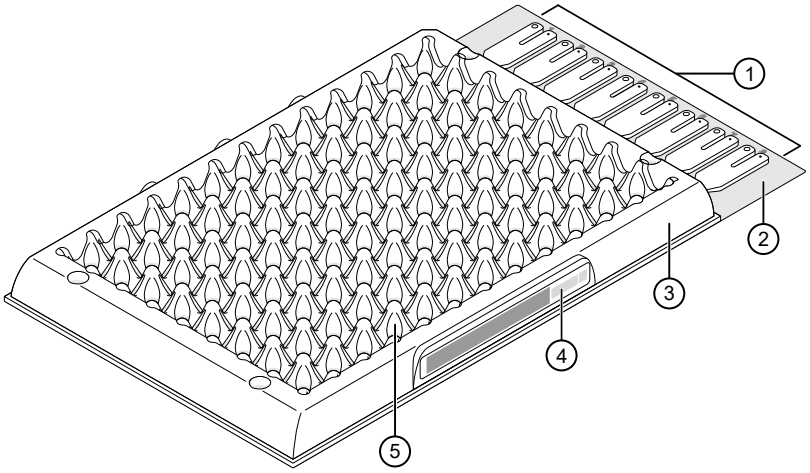
Guidelines for preparation of a card

- Keep the card protected from light and stored as indicated until ready for use. Excessive exposure to light may affect the fluorescent probes of the dried-down assays in the card.
- Before removing the card from its packaging:
  - Prepare each sample-specific PCR reaction mix.
  - Allow the card to reach room temperature.
- Load each fill reservoir with 100 µL of sample-specific PCR reaction mix.
  - Each fill reservoir contains a single sample as determined by the card layout.
  - The 100–µL volume ensures adequate filling of each reaction well. Volumes smaller than 100 µL result in insufficiently filled cards.
- Do not allow the micropipette tip to contact the coated foil beneath the fill port.

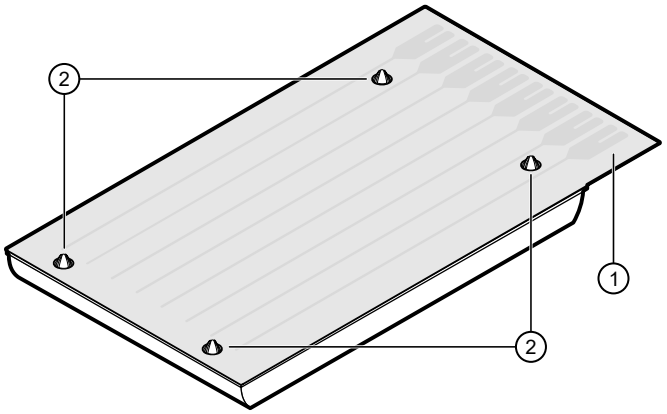
- Load the card with PCR reaction mix *before* centrifuging the card.
  During centrifugation, the PCR reaction mix resuspends the dried-down assays in each well of the card. Adding sample after centrifuging disrupts the assay layout of the card.
- Run the card within the time allowed by the Master Mix.
- If the card is not run immediately, protect it from light and store at 2-8°C.

TaqMan™ Array Card diagram

A TaqMan™ Array Card includes 8 fill reservoirs and 384 reaction wells.

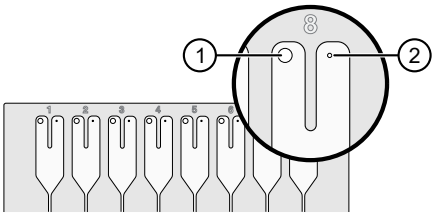


① Fill reservoirs (8 total)
② Fill reservoir strip
③ Array card carrier
④ Array card barcode
⑤ Reaction well (384 total)



① Foil
② Array card feet

The fill reservoir includes a fill port and a vent port. Use the fill port to load PCR reaction mix into the card.



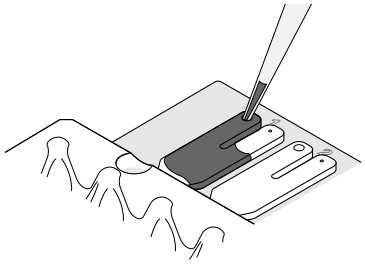
① Fill port
② Vent port

Load the PCR reaction mix

Before removing the card from its packaging:

- Prepare each sample-specific PCR reaction mix.
- Allow the card to reach room temperature.

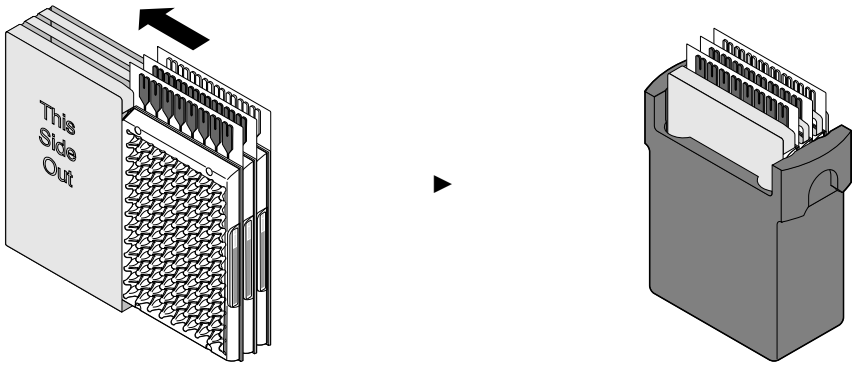
1. Carefully remove the card from its packaging.
2. Place the card on the benchtop with its foil-side down.
3. Load 100 µL of the sample-specific PCR reaction mix into a micropipette.
4. Hold the micropipette in an angled position, then place the tip into a fill port of the card.
5. Slowly dispense the entire volume of reaction mix so that it sweeps in and around the fill reservoir toward the vent port.



Centrifuge the card

1. Load the cards into the centrifuge buckets.
   a. Place the bucket on the benchtop with its label facing the front of the bench.
   b. Insert the cards into the card holder, ensuring that:
      - The fill reservoirs extend upwards out of the card holder.
      - The reaction wells face the label-side of the card holder.
   c. Insert blank balance cards into any empty positions of the card holder. All three positions in the card holder must be filled.

d. Place the loaded card holder into the bucket so that the card holder label faces the front of the bucket.



2. Configure the centrifuge using its front-panel controls.

   a. Set the bucket type to **15679**.

   b. Set the following parameters according to the control panel type.

Parameter	EASYSet (touchpad-operated)	QUIKSet (knob-operated)
Increasing ramp rate	9	3
Decreasing ramp rate	9	—
Rotational speed	1,200 rpm (331 × *g*)	1,200 rpm
Centrifugation time [1]	1 minute	1 minute

[1] You will centrifuge the cards twice, each time for 1 minute (see step 4).

IMPORTANT! A speed that is set too high can deform the card.

3. Load the buckets into the centrifuge.

   a. Press ⏏ on the centrifuge control panel to open the centrifuge cover.

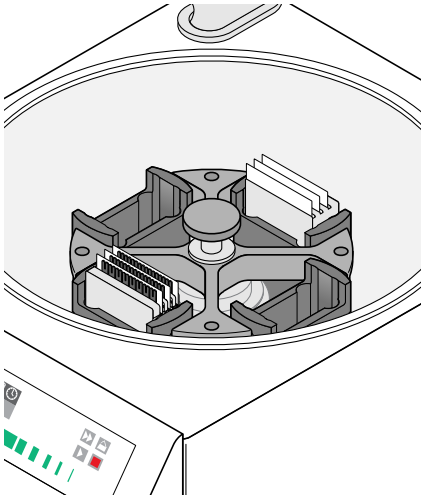
   b. Place each loaded bucket onto an open rotor arm of the centrifuge.

   Ensure that each bucket can swing easily within its slotted position on the rotor arm.

   c. If there are empty rotor arms, prepare buckets with blank balance cards as described in step 1. Place the balance buckets onto the rotor arms.

   The rotor must be evenly loaded and opposing buckets must hold the same weight.

   d. Close the centrifuge cover.



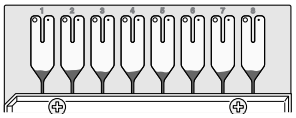
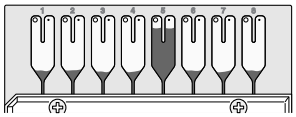
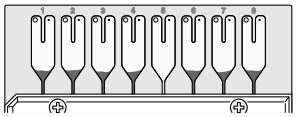
Centrifuge is properly loaded and balanced.

4. Run the centrifuge.
   a. Press ▶.
      The centrifuge will start, then automatically stop after 1 minute.
   b. Repeat substep 4a so that the cards are centrifuged for a total of two, consecutive, 1-minute centrifugations.

      IMPORTANT! Do not centrifuge the cards continuously for 2 minutes. The ramping up in speed during the *two, consecutive 1-minute* centrifugations is important for proper filling.

5. Remove the cards from the centrifuge.
   a. Press ⏏.
   b. Remove the buckets from the centrifuge, then remove the card holders from the buckets.
   c. Remove each card from the card holder by lifting it gently by the card carrier sides.

6. Examine the cards for proper filling.
   When properly filled, the remaining volumes of PCR reaction mix are consistent reservoir to reservoir.

Consistent filling	Inconsistent filling	
		

Seal the card

The TaqMan™ Array Card Sealer isolates the wells of an array card after it is loaded with PCR reaction mix and centrifuged. The sealer uses a precision stylus assembly (under the carriage) to seal the main fluid distribution channels of the array card.

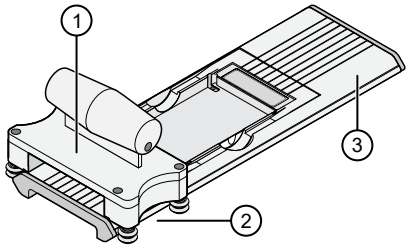
Note: In some documents, the TaqMan™ Array Card Sealer is referred to as a Stylus Staker.

1. Position the TaqMan™ Array Card Sealer and its carriage before inserting a card.

   a. Place the sealer on a benchtop that is approximately waist-high so that the carriage can be easily maneuvered.

   b. Position the sealer with the carriage starting position toward the front of the bench.

   Ensure that the engraved arrows on the sealer point away from you.



① Carriage
② Carriage starting position
③ Carriage ending position

   c. Ensure that the carriage for the sealer is at the starting position.

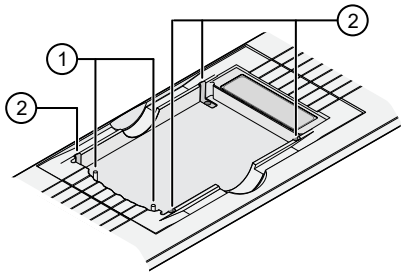
   IMPORTANT! Do not insert a filled card into the sealer if the carriage is *not* in its starting position. The card will be irreparably damaged if the carriage is moved backwards across the card towards the starting position.

2. Insert a filled, centrifuged card into the sealer.

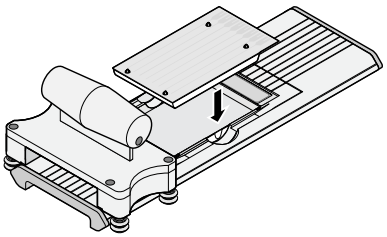
   a. Hold the card with its foil-side up.

   b. Orient the card over the sealer with the fill reservoirs of the card toward the ending position.

   c. Align the rear pin grooves of the card to the alignment pins of the sealer.



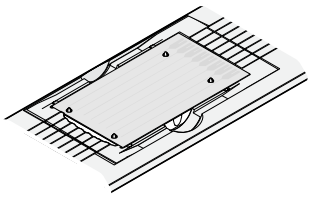
① Alignment pins
② Spring clips



   d. Gently place the card on top of the sealer.

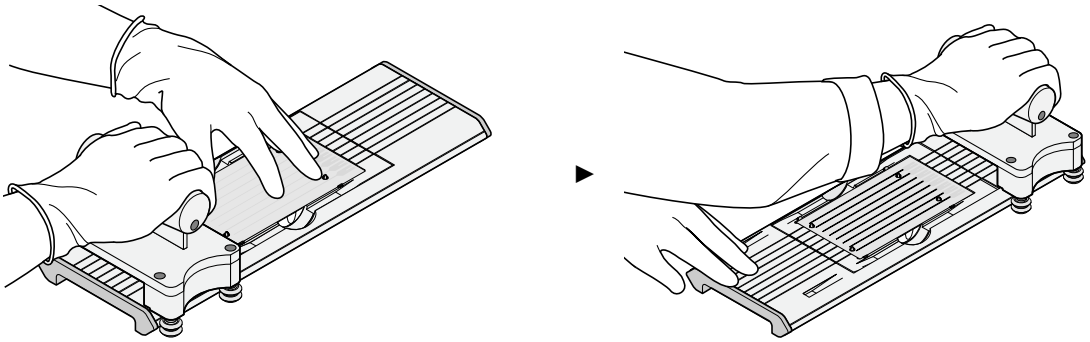
e. Gently push the card until it is seated securely in the sealer.

When properly seated, the foil surface of the card is level with the base of the sealer and the spring clips hold the card securely in place.



3. Slowly and steadily push the carriage across the sealer in the direction of the engraved arrows.

Push the carriage across the entire length of the card until the carriage meets the mechanical stops at the ending position.



IMPORTANT!

- Do not use excessive force or speed when pushing the carriage across the card.
- Do not move the carriage back across the card. Leave the carriage at the ending position while removing the card from the sealer.

4. Remove the sealed card from the sealer by grasping the sides of the card and lifting it off.

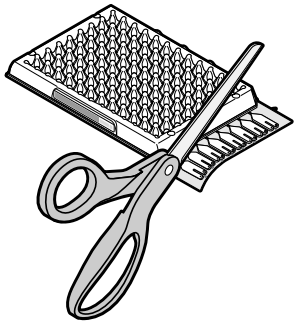
Use the thumb slot in the middle of the sealer to access the card.

5. Examine the card for proper sealing.

Note: When properly sealed, the indentations from the sealer carriage align with the main channels of the card.

If the indentations do not align or if the foil is damaged, do not use the card.

6. Use scissors to trim the fill reservoir strip from the card. Use the edge of the card carrier as a guide.



IMPORTANT! Completely remove the fill reservoir strip. Any remaining plastic that extends beyond the card edge can prevent the card from seating properly on the sample block and can affect amplification.

Correct trim	Incorrect trim
	

The card is now ready to run on the instrument.

Run the card within the time allowed by the Master Mix.

C TaqMan™ assay pools included in the TaqMan™ Microbial Array Specialty Card, Study Name "Wound+ABR Expanded Panel"—component assay IDs

The following tables list component assay IDs within each pool. To order the correct pool, use the pool assay IDs listed in page 6.

Table 11 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
7	*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
14	*Klebsiella oxytoca*	Gram-negative	Ba04932079_s1
	Klebsiella pneumoniae	Gram-negative	Ba04932083_s1
16	*Proteus mirabilis*	Gram-negative	Ba04932076_s1
	Proteus vulgaris	Gram-negative	Ba04932082_s1
21	*Streptococcus agalactiae*	Gram-positive	Ba04646276_s1
	Streptococcus anginosus	Gram-positive	Ba07922557_s1
	Streptococcus dysgalactiae	Gram-positive	Ba07921957_s1
24	*Peptoniphilus asaccharolyticus*	Gram-positive	Ba07922560_s1

Table 11 Assay pools for wound microbiota targets—component assay IDs *(continued)*

#[1]	Pathogen	Bacterial gram strain	Assay ID
24	*Peptoniphilus harei*	Gram-positive	Ba07922183_s1
	Peptoniphilus ivorii	Gram-positive	Ba07922563_s1

[1] From Table 1 on page 6.

Table 12 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	Ba04646116_s1
			Ba04646119_s1
			Ba04646131_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
		blaVIM	Ba07922583_s1
8	Extended-spectrum beta-lactamases 1	blaCTX-M	Ba04646142_s1
			Ba04646149_s1
			Ba07922581_s1
			Ba04646127_s1

Table 12 Assay pools for antibiotic resistance targets—component assay IDs *(continued)*

#[1]	Antibiotic	Gene family	Assay ID
10	Extended-spectrum beta-lactamases 3	blaGES	Ba04646151_s1
		blaPER	Ba04646140_s1
		blaVEB	Ba04646153_s1
12	Macrolides 1	ere(B)	Ba07319981_s1
		mef(A)	Ba07922579_s1
		mph(A)	Ba07922580_s1
		msr(A)	Ba07922570_s1
13	Macrolides 2	erm(A)	Ba07922565_s1
			Ba04646137_s1
		erm(B)	Ba04230913_s1
		erm(C)	Ba07319994_s1
14	Methicillin	mecA	Ba04230908_s1
		mecC	Ba07319993_s1
15	Nitromidazole	nimB	Ba07319983_s1
		nimD	Ba07320001_s1
		nimE	Ba07319999_s1
		nimJ	Ba07320000_s1
16	Quinolone 1	qnrB	Ba07922569_s1
			Ba07922571_s1
			Ba07922572_s1
17	Quinolone 2	qnrA	Ba07922584_s1
		qnrS	Ba04646145_s1
18	Sulfonamide	sul1	Ba07319988_s1
		sul2	Ba07320003_s1
19	Tetracycline	tet(A)	Ba07922566_s1
		tet(B)	Ba07921939_s1
		tet(M)	Ba04230915_s1
		tet(S)	Ba07319979_s1

Table 12 Assay pools for antibiotic resistance targets—component assay IDs *(continued)*

#[1]	Antibiotic	Gene family	Assay ID
20	Trimethoprim	dfrA1	Ba07319989_s1
		dfrA5	Ba07319986_s1
		dfrA12	Ba07922567_s1
		dfrA17	Ba07922575_s1
21	Vancomycin	vanA	Ba04646147_s1
		vanB	Ba04646150_s1

[1] From Table 2 on page 7.

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Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, 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

Documentation and support

Related documentation

Document	Publication Number
MagMAX™ Prime Viral/Pathogen NA Isolation Kit and Accessories User Guide	MAN0029683
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.3) Maintenance and Administration Guide	4489821
QuantStudio™ 12K Flex Real–Time PCR System: Multi-Well Plates and Array Card Experiments User Guide	4470935
QuantStudio™ 12K Flex Real–Time PCR System v1.4 Maintenance and Administration Guide	4470689
Thermo Scientific™ KingFisher™ Flex User Manual	N07669
TaqMan™ Universal Extraction Control Organism (B. atrophaeus) Product Information Sheet	MAN0018535
TrueMark™ Xeno Control, Kanamycin Resistance Product Information Sheet	MAN0026587

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

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

Limited product warranty

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