


# TaqMan™ Controls with TaqMan™ Array Cards

Pub. No. MAN0025035 Rev. A.0

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

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

For detailed instructions to handle and prepare TaqMan™ Array Cards, see the *TaqMan™ Gene Expression Assays User Guide—TaqMan™ Array Cards* (Pub. No. 4400263).

## Product description

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

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

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

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

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

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

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

## Required materials

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](https://www.fisherscientific.com) or another major laboratory supplier.

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

Item	Source
<b>Real-time PCR instrument, one of the following:</b>	
The instrument must be configured with the TaqMan™ Array Card block and heated cover.	
QuantStudio™ 7 Pro Real-Time PCR System	Contact your local sales office
QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 12K Flex Real-Time PCR System	
ViiA™ 7 Real-Time PCR System	
7900HT Fast Real-Time PCR System	

Item	Source
<b>Equipment</b>	
Centrifuge with custom buckets and card holders, one of the following: <ul style="list-style-type: none"> <li>• Sorvall™ centrifuge</li> <li>• Megafuge™ centrifuge</li> <li>• Multifuge™ centrifuge</li> </ul> See the <b>Resources</b> section at <a href="http://thermofisher.com/taqmanarrays">thermofisher.com/taqmanarrays</a> for a list of compatible centrifuges, rotors, and buckets.	Contact your local sales office
TaqMan™ Array Card Sealer (Referred to as Stylus Staker in some documents)	Contact your local sales office
Blank balance TaqMan™ Array Cards (Included with the instrument block upgrade / installation kit)	Contact your local sales office
Microcentrifuge	MLS
Vortex mixer	MLS
(Optional) Eppendorf™ MixMate™ (shaker)	Fisher-Scientific 21-379-00
Pipettes	MLS
Micropipettes	MLS
<b>Tubes, plates, and other consumables</b>	
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	<a href="#">AM12450</a>
Nonstick, RNase-Free Microcentrifuge Tubes, 0.5 mL	<a href="#">AM12350</a>
<b>Reagents</b>	
TaqMan™ Fast Advanced Master Mix	4444556
TE Buffer	<a href="#">12090015</a>
Nuclease-free Water	<a href="#">AM9939</a>

## Guidelines

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

## Dilute the controls

Dilute the control according to one of the following procedures, depending on the concentration of the control.

- Dilute the control ( $1 \times 10^5$  copies/ $\mu\text{L}$ ) to the recommended concentration of  $4 \times 10^3$  copies/ $\mu\text{L}$ , according to the table below.

Component	Volume
Control at $1 \times 10^5$ copies/ $\mu\text{L}$	10 $\mu\text{L}$
TE Buffer	240 $\mu\text{L}$
<b>Total volume of control at <math>4 \times 10^3</math> copies/<math>\mu\text{L}</math></b>	<b>250 <math>\mu\text{L}</math></b>

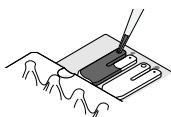
- Dilute the control ( $5 \times 10^7$ ) in a serial dilution to a concentration of  $1 \times 10^5$  copies/ $\mu\text{L}$ , according to the tables below.

Component	Volume
Control at $5 \times 10^7$ copies/ $\mu\text{L}$	10 $\mu\text{L}$
TE Buffer	40 $\mu\text{L}$
<b>Total volume of control at <math>1 \times 10^7</math> copies/<math>\mu\text{L}</math></b>	<b>50 <math>\mu\text{L}</math></b>

Component	Volume
Control at $1 \times 10^7$ copies/ $\mu\text{L}$ (from previous table)	10 $\mu\text{L}$
TE Buffer	990 $\mu\text{L}$
<b>Total volume of control at <math>1 \times 10^5</math> copies/<math>\mu\text{L}</math></b>	<b>1,000 <math>\mu\text{L}</math></b>

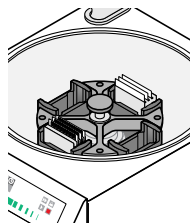
## Workflow

### Prepare and run a TaqMan™ Array Card



#### Load each port with Reaction Mix

Contains TaqMan™ Fast Advanced Master Mix, Nuclease-free Water, and sample.



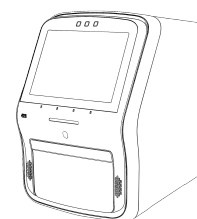
#### Centrifuge the TaqMan™ Array Card

Centrifuge to fill the wells of the array card.



#### Seal the TaqMan™ Array Card

Seal the array card to isolate each well of the array card.



#### Set up and run the real-time PCR instrument

## Guidelines to prepare a TaqMan™ Array 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.

## Prepare and run a TaqMan™ Array Card

For detailed instructions for handling TaqMan™ Array Cards, see *TaqMan™ Gene Expression Assays User Guide—TaqMan™ Array Cards* (Pub. No. 4400263).

Download the plate file for the array card at [thermofisher.com/taqmanfiles](http://thermofisher.com/taqmanfiles).

1. Allow the card to equilibrate to room temperature.
2. Gently mix the bottle of TaqMan™ Fast Advanced Master Mix.
3. Prepare a Reaction Mix for each port according to the following table.

Component	Volume per port
TaqMan™ Fast Advanced Master Mix	55.0 µL
Nuclease-free Water	49.5 µL
Sample or Nuclease-free Water <sup>[1]</sup>	5.5 µL
<b>Total volume per port</b>	<b>110 µL</b>

<sup>[1]</sup> Nuclease-free Water for a No-Template Control.

4. Fill each port with 100 µL of the Reaction Mix prepared in step 3.
5. Centrifuge the card at 1,200 rpm (301 × g) for 1 minute.
6. Repeat step 5.

---

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

---

7. Seal the card using TaqMan™ Array Card Sealer.
8. Load the array 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	Temperature	Time	Ramp rate	Number of cycles
UNG incubation	50°C	2 minutes	1.75°C/second	1
Activation	92°C	10 minutes	1.75°C/second	1
Denaturation	95°C	1 second	1.75°C/second	40
Annealing/extension	60°C	20 seconds	1.83°C/second	

9. Start the run.

## Related documentation

Document	Publication number
<i>TaqMan™ Gene Expression Assays User Guide—TaqMan™ Array Cards</i>	4400263
<i>TaqMan™ Custom DNA Controls Product Information Sheet</i>	MAN0024963
<i>TaqMan™ Comprehensive Microbiota Control Product Information Sheet</i>	MAN0024964
<i>Respiratory Tract Microbiota Profiling Experiments using TaqMan™ Array Cards Application Guide</i>	MAN0017951
<i>Respiratory Tract Microbiota Profiling Experiments v2 using TaqMan™ Array Cards Application Guide</i>	MAN0019507
<i>Urinary Tract Microbiota Profiling Experiments Application Guide</i>	MAN0017750
<i>Vaginal Microbiota Profiling Experiments Application Guide</i>	MAN0015669

## Customer and technical support

Visit [thermofisher.com/support](https://www.thermofisher.com/support) for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

## Limited product warranty

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



Thermo Fisher Scientific GENEART GmbH | Im Gewerbepark B35 | 93059 Regensburg | Germany

For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

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

**DISCLAIMER:** TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

**Revision history:** Pub. No. MAN0025035

Revision	Date	Description
A.0	31 March 2021	New document for the TaqMan™ Comprehensive Microbiota Controls and the TaqMan™ Custom DNA Controls with TaqMan™ Array Cards.

**Important Licensing Information:** These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

©2021 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a registered trademark of Roche Molecular Systems, Inc., used under permission and license. Eppendorf and MixMate are trademarks of Eppendorf AG.