applied biosystems

TrueMark™ Custom Plates

USER GUIDE

96-well Fast (0.1-mL) TrueMark™ Custom Plates
96-well Standard (0.2-mL) TrueMark™ Custom Plates
384-well TrueMark™ Custom Plates

Catalog Numbers A55017, A55018, A55019, A55020, A55021, A55022

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

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

Revision history: Pub. No. MAN0025767

Revision	Date	Description
E.0	5 August 2022	Reference and SKU were added to include TrueMark™ Amplification Control I, low conc.
D.0	13 May 2022	• Updates made throughout the user guide to update references to TrueMark™ Custom Plates. • Updates made to reference controls and SKUs in "Product description" on page 4. • Updates made to numbers of assays in "Contents and storage" on page 5. • Materials were revised in "Required materials and equipment not supplied" on page 5. • The instructions for preparing the PCR plate were revised (see "Prepare the TrueMark™ Custom Plate" on page 8). • The plate preparation steps were revised and instructions were added to use the MicroAmp™ Optical Film Compression Pad (see "Set up and run the real-time PCR instrument" on page 10). • The optional controls were revised to reference positive control, negative control, and "TaqMan™ Universal Extraction Control Organism (B. atrophaeus)" on page 12. • References were updated (see "Related documentation" on page 17).
C.0	15 December 2021	• The instructions for preparing the PCR plate were revised (see "Prepare the TrueMark™ Custom Plate" on page 8). • The thermal cycling conditions were revised to note that the reverse transcription step is for RNA panels only (see "Set up and run the real-time PCR instrument" on page 10). • Reference of TE Buffer added to "Required materials and equipment not supplied" on page 5.
B.0	10 November 2021	• Removed preamplification. • Updated to include QuantStudio™ 5 Dx Real-Time PCR System and QuantStudio™ 7 Pro Real-Time PCR Systemwith QuantStudio™ Design and Analysis v2.6. • Updated to include TaqPath™ 1-Step Multiplex Master Mix (No ROX™). • Updated the following: – "Contents and storage" on page 5 – "Required materials and equipment not supplied" on page 5 – "Workflow" on page 7 – "Guidelines for isolating high-quality RNA or DNA" on page 8 – "Set up and run the real-time PCR instrument" on page 10 – "Analyze the results" on page 11 – "Related documentation" on page 17
A.0	8 October 2021	New document for TaqMan™ Infectious Disease Assays—TaqMan™ Array Plates.

Contents

1 Product information

IMPORTANT! It is the responsibility of laboratories to design and validate their own experimental design and analysis parameters.

Product description

TrueMark™ Custom Plates are a collection of duplex assays that detect microbes and internal controls in the same reaction. They are available as fully customizable Custom TaqMan™ Array Plates.

TaqMan™ Array Plates can be ordered using any of the following SKUs:

- A55017
- A55018
- A55019
- A55020
- A55021
- A55022

The following controls are used:

- Ribonuclease P (RNase P)—an endogenous sample collection control for the respiratory tract infection (RTI), sexually transmitted infection (STI), lesion, vaginal, and genital infection panels.
- *Bacillus atrophaeus* (TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*))—a process control for the urinary tract infection panel (UTI).

For a current list of available species and assays, use the assay search tool at **https://www.thermofisher.com/microbe-detection/taqman/query/**.

The assays are designed for use in real-time PCR using a variety of compatible instruments (see Chapter 2, “Methods”). For detailed information about TrueMark™ Custom Plates, see Appendix A, “Supplemental information”.

Contents and storage

TrueMark™ Custom Plates are orderable as customizable plates. The available plate configurations are shown in the following table.

Table 1 TrueMark™ Custom Plates

Format	Number of Assays	Number of samples	Storage[1]	Ordering information
TrueMark™ Custom Plate (96 wells)	Up to 8	12	15–30°C[2]	Contact your local sales office
TrueMark™ Custom Plate (384 wells)	Up to 24	16	15–30°C[2]	

[1] See packaging for expiration date.

[2] Shipped at ambient temperature. See thermofisher.com/ambientshippping.

Required materials and equipment 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.

Table 2 Recommended products for isolation of RNA/DNA

Item	Source
MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit (RNA/DNA)	A42356

Table 3 Master mixes

Item	Source
TaqPath™ 1-Step Multiplex Master Mix (No ROX™)	A28522, A28523

Table 4 Other materials and equipment required for the workflow

Item	Source
Real-time PCR instrument, one of the following:	
(Recommended) QuantStudio™ 5 Real-Time PCR System	Contact your local sales office
(Recommended) QuantStudio™ 5 Dx Real-Time PCR System	
(Recommended) QuantStudio™ 7 Pro Real-Time PCR System	
Software:	
QuantStudio™ Design and Analysis v2.6	thermofisher.com/qpcrsoftware

Table 4 Other materials and equipment required for the workflow *(continued)*

Item	Source
QuantStudio™ Real-Time PCR Software	**thermofisher.com/qpcrsoftware**
Equipment:	
Centrifuge, with adapter for 96-well plates	MLS
Microcentrifuge	MLS
Vortex mixer	MLS
(Optional) Digital Microplate Shaker	88882005 or 88882006
Pipettes	MLS
Tubes, plates, and other consumables:	
Tubes, plates, and film	**thermofisher.com/plastics**
MicroAmp™ Optical Adhesive Film	4311971
MicroAmp™ Optical Film Compression Pad	4312639
Aerosol-resistant barrier pipette tips	MLS
Disposable gloves	MLS
Reagents:	
Nuclease-free water	AM9930
PBS (1X), pH 7.4	10010023
TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*)	A39180
TrueMark™ Amplification Control I, low conc	A55699
TE Buffer	12090015

Workflow

TrueMark™ Custom Plates

Guidelines for isolating high-quality RNA or DNA (page 8)

Prepare the TrueMark™ Custom Plate (page 8)

Set up and run the real-time PCR instrument (page 10)

Analyze the results (page 11)

2 Methods

Guidelines for isolating high-quality RNA or DNA

- See Table 2 for recommended RNA and DNA isolation kits. The real-time PCR procedure requires 5 µL of extracted RNA or DNA per reaction.

Guidelines for real-time PCR

- Follow best practices when preparing or performing PCR (see page 13).
- Prepare the real-time PCR reactions in an area free of artificial templates and siRNA transfections. High-copy-number templates can easily contaminate the real-time PCR reactions.
- Configure run documents according to the instructions provided in the real-time PCR instrument user documents.
- Keep the plate 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 plate.
- Thaw the TaqPath™ 1-Step Multiplex Master Mix (No ROX™) on ice.

Prepare the TrueMark™ Custom Plate

Note: For TrueMark™ Custom Plates with more than 8 targets, the nucleic acid extraction procedure may not yield sufficient volume for 5 µL of RNA or DNA per reaction. For example, a 24-target plate requires ~140 µL of sample to cover all targets (with overage). For these plates, you may choose to either increase elution buffer volume up to 180 µL to cover all targets or pool multiple extractions. If

elution buffer volume increase is required, the optimal volume must be determined empirically for your samples.

1. *Plates with >8 targets:* If necessary, increase elution buffer volume up to 180 µL to cover all targets or pool multiple extractions (see note above).
2. Mix the TaqPath™ 1-Step Multiplex Master Mix (No ROX™) by inverting the bottle.
3. Prepare the appropriate volume of master mix and nuclease-free water as shown in the following tables, depending on the plate type and number of reactions.

Table 5 96-well Fast (0.1-mL) plate or 384-well plate

Component	Volumes[1]			
	1 well	8 wells	32 wells	96 wells
Master Mix (4X)	2.5 µL	22.5 µL	90 µL	270 µL
Nuclease-free water	2.5 µL	22.5 µL	90 µL	270 µL
Total volume	**5 µL**	**45 µL**	**180 µL**	**540 µL**

[1] Volumes for multiple reactions include a 12.5% overage.

Table 6 96-well Standard (0.2-mL) plate

Component	Volumes[1]			
	1 well	8 wells	32 wells	96 wells
Master Mix (4X)	5 µL	45 µL	180 µL	540 µL
Nuclease-free water	10 µL	90 µL	360 µL	1080 µL
Total volume	**15 µL**	**135 µL**	**540 µL**	**1,620 µL**

[1] Volumes for multiple reactions include a 12.5% overage.

4. Remove the plate from its packaging, centrifuge at 2,000 x *g* for 2 minutes, then remove the plate cover.
5. Add the prepared master mix and nuclease-free water to the appropriate wells of the plate.
6. Add 5 µL of extracted RNA or DNA to each reaction well of the plate.
7. Seal the plate with MicroAmp™ Optical Adhesive Film, then vortex each corner and center for approximately 10 seconds at maximum speed.
8. Centrifuge the plate at 1,400 x *g* for 2 minutes, to collect the contents to the bottom of the wells.

IMPORTANT! Run the plate immediately.

Set up and run the real-time PCR instrument

See the appropriate instrument user guide for detailed instructions to program the thermal-cycling conditions and run the plate.

Note: The instrument must be configured with the block appropriate for the plate type (96-well Fast, 96-well Standard, or 384-well plate).

1. Set up samples and targets on the real-time PCR instrument or software.

   Note: Ensure the target is in the FAM channel and the control in the JUN channel. Ensure the real-time PCR instrument is calibrated with the JUN channel.

2. Set up the thermal cycling conditions for your instrument.

   Table 7 TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (QuantStudio™ systems with standard cycling mode)

Step	Temperature	Ramp rate	Time	Number of cycles
UNG incubation	25°C	1.6°C per second	2 minutes	1
For RNA panels only: Reverse transcription	53°C		10 minutes	1
Preincubation	85°C		10 minutes	1
Activation	95°C		2 minutes	1
Denaturation	95°C		3 seconds	40
Anneal / extension	60°C		30 seconds	

3. Set the reaction volume appropriate for the plate type.
   - 96-well Fast (0.1-mL) plate: 10 µL per well
   - 96-well Standard (0.2-mL) plate: 20 µL per well
   - 384-well plate: 10 µL per well

4. Load the prepared plate into the real-time PCR instrument.

   Note: When prompted, confirm that the **Passive Reference** is set to **None**.

5. *Perform the following step only when using a 0.2-mL block with the QuantStudio™ 5 Real-Time PCR System.* Place a MicroAmp™ Optical Film Compression Pad gray side down on the surface of the RT-PCR reaction plate, to ensure a proper seal between the thermal cycler and the adhesive film.

   IMPORTANT!
   - Be careful to place the compression pad with the brown side up and the gray side down, centered on top of the plate.
   - Ensure the compression pad is free from wrinkles and signs of deterioration prior to use.
   - Discard compression pad after 20 uses.

6. Start the run.

Analyze the results

Use QuantStudio™ Design and Analysis v2.6 for data analysis or export files directly from the instrument QuantStudio™ Real-Time PCR Software. For more information about using the software, see “Related documentation” on page 17.

Note: For detailed information about data analysis, see the appropriate documentation for your instrument.

1. In the QuantStudio™ Design and Analysis v2.6 or QuantStudio™ Real-Time PCR Software home screen, open the data file (EDS).

2. In the open data file, click **Actions ▸ Save As**, then save the data file with a new name.

   Note: QuantStudio™ Design and Analysis v2.6 requires data files created on the following instruments to be saved as a new data file:
   - QuantStudio™ 5 Real-Time PCR System
   - QuantStudio™ 5 Dx Real-Time PCR System

3. In the analysis settings, select automatic baseline with a start cycle of 5 and an end cycle of auto, or select according to lab validation.

4. Set the appropriate threshold values for each target, as validated by your laboratory.

   IMPORTANT! Do not use automatic threshold values.

5. Determine C_q cutoff values for each target for samples and controls.

   Note: QuantStudio™ Design and Analysis Software v2 reports C_q values instead of C_t values. The C_q values are equivalent to C_t values.

6. Analyze results according to analysis, interpretation, and QC parameters, as validated by your laboratory.

Contact Support for more information.

A Supplemental information

Optional controls

Positive and negative controls

The following controls are recommended for each assay:

Control	Description
TrueMark™ Amplification Control I, low conc	Is added to a single well in the real-time PCR plate. Monitors real-time PCR reaction setup and reagent integrity. Can be ordered seperately, which includes all the targets in the TrueMark™ panels. Can be diluted before adding to the PCR reactions. The optimal concentration can be decided by the lab.
Negative (extraction) control	A negative control on the extraction sample plate that contains water. The extracted negative control sample is run on the real-time PCR plate to monitor cross contamination during reaction setup.

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

TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*) (Cat. No. A39180), contains three vials of lyophilized *Bacillus atrophaeus*, for use as a process control for cell lysis and nucleic acid recovery. This control is used with the proprietary TaqMan™ Assay for *Bacillus atrophaeus* sequences.

Like other gram-positive bacteria, *Bacillus atrophaeus* has thick cell walls that can be difficult to lyse. This characteristic makes *Bacillus atrophaeus* 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.

The following *B. atrophaeus* dilution and the spike in process instructions are recommended:

Vortex for 30 seconds, until the pellet is resuspended. Make the suitable number of aliquots and stored at –20°C. Perform further dilution to obtain the working concentration of 5×10^4 copies/µL.

10 uL of the control is added to urine samples in each extraction well at the start of extraction.

Best practices for PCR and RT-PCR experiments

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

Detect fluorescent contaminants

Fluorescent contaminants can generate false positive results. To help detect these contaminants, we recommend including a no-amplification control reaction that contains sample, but no master mix.

After PCR, if the absolute fluorescence of the no-amplification control is greater than the fluorescence of the no template control (NTC), fluorescent contaminants may be present in the sample or in the heat block of the real-time PCR instrument.

Algorithms for data analysis

Table 8 Algorithm recommendations for TrueMark™ Custom Plates

Algorithm	Recommendation
Threshold (C_t)	• Recommended for data analysis.
Relative threshold (C_{rt})	• *(Optional)* Use for data analysis. • Use to troubleshoot unexpected results. • Use to correct a variable baseline, which can be due to dried-down assays on the plate being reconstituted at different rates.

The relative threshold algorithm is available in the Relative Quantification application on Thermo Fisher™ Connect (**thermofisher.com/connect**).

B

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, see the “Documentation and Support” section in this document.

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.

Biological hazard safety

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.

- 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
- 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
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf

C Documentation and support

Related documentation

Document	Pub. No.
TaqPath™ 1-Step Multiplex Master Mix User Guide	MAN0014269
TaqPath™ 1-Step Multiplex Master Mix Quick Reference	MAN0014389
TaqPath™ 1-Step Multiplex Master Mix Product Information Sheet	MAN0014069, 100033995
TaqMan™ Universal Extraction Control Organism (B. atrophaeus) Product Information Sheet	MAN0018535
QuantStudio™ 5 Dx Real-Time PCR Instrument Maintenance and Administration User Guide	100042186
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
QuantStudio™ Design and Analysis Desktop Software User Guide	MAN0010408
QuantStudio™ 6 Pro Real-Time PCR System and QuantStudio™ 7 Pro Real-Time PCR System User Guide	MAN0018045
QuantStudio™ Design and Analysis Software v2 User Guide	MAN0018200

Customer and technical support

Visit **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**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.