


TaqMan™ Microbe Detection and Antibiotic Resistance Assays

Catalog Numbers A50133, A50136, A50137

Pub. No. MAN0025572 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.

Note: For safety and biohazard guidelines, see the “Safety” appendix in the *TaqMan™ Gene Expression Assays User Guide—single-tube assays* (Pub. No. 4333458). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This document is intended as a benchtop reference for experienced users. For documents with detailed instructions and troubleshooting, see “Related documentation” on page 4.

Contents and storage

Assay	Cat. No.	Volume	Concentration	Storage
TaqMan™ Microbe Detection Assay or Antibiotic Resistance Assay, Made-to-Order	A50133	360 µL	20X	–25°C to –15°C
TaqMan™ Antibiotic Resistance Assay, Inventoried	A50136	250 µL	20X	
TaqMan™ Microbe Detection Assay, Inventoried	A50137	250 µL	20X	

Procedural guidelines

Guidelines for nucleic acid isolation

- For detailed instructions about nucleic acid isolation for microbial experiments using the MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit, see the appropriate resources that are listed in “Related documentation” on page 4.
- A 400-µL sample input yields approximately 50 µL of purified sample. Depending on the number of assays to be tested, multiple 400-µL sample aliquots may be needed.

Guidelines for real-time PCR

- For amplification of RNA targets, use TaqPath™ 1-Step RT-qPCR Master Mix, CG (Cat. No. [A15299](#)).
- For amplification of RNA targets, use the 1-step RT-PCR procedure (see “Perform 1-step RT-PCR” on page 2).
- For amplification of cDNA targets, use TaqMan™ Fast Advanced Master Mix, No UNG (Cat. No. [A44359](#)).
- For amplification of cDNA targets, use the PCR procedure (see “Perform RT-PCR” on page 3).
- Use purified, nondegraded total nucleic acid that is dissolved in PCR-compatible buffer.
- Ensure that the input nucleic acid is free of RNase activity and inhibitors of reverse transcription (RT) and PCR.
- To maximize sensitivity, prepare each reaction so that approximately half of the reaction volume is purified total nucleic acid.
- Protect the assays from light and store as indicated until ready for use. Excessive exposure to light can negatively affect the fluorescent probes of the assays.
- (Optional) Run technical replicates in triplicate to identify outliers.

Perform 1-step RT-PCR

Use the 1-step RT-PCR procedure for amplification of RNA targets.

Prepare the 1-step RT-PCR reactions

Thaw the microbial RNA samples on ice. Resuspend the samples by inverting the tube, then gently vortexing.

1. Gently mix the bottle of TaqPath™ 1-Step RT-qPCR Master Mix, CG.
2. Combine the following components for the number of required reactions, plus 10% overage.

IMPORTANT! For optimal results, prepare the reaction plate on ice.

Component	Volume per well	
	96-well, 0.2-mL plates	384-well plates or 96-well 0.1-mL plates
TaqPath™ 1-Step RT-qPCR Master Mix, CG (4X)	5.0 µL	2.5 µL
Assay (20X)	1.0 µL	0.5 µL
Nuclease-free water	4.0 µL	2.0 µL
Total PCR Reaction Mix volume	10.0 µL	5.0 µL

3. Vortex the PCR Reaction Mix, then centrifuge briefly.
4. Combine the following components in each well of an optical reaction plate.

Component	Volume per well	
	96-well, 0.2-mL plates	384-well plates or 96-well 0.1-mL plates
PCR Reaction Mix (from step 2)	10.0 µL	5.0 µL
Extracted microbial nucleic acid sample <i>or</i> elution buffer for NTC	10.0 µL	5.0 µL
Total volume	20.0 µL	10.0 µL

Note: Adjust the volume of nuclease-free water in the PCR Reaction Mix for a larger volume of RNA.

5. Seal the plate with a MicroAmp™ Optical Adhesive Film, then vortex briefly to mix the contents.
6. Centrifuge the plate briefly to collect the contents at the bottom of the wells.

IMPORTANT! Vortexing and centrifuging are required for proper mixing of the reaction components.

IMPORTANT! Run the plate within 2 hours of preparation, or store the plate at 2–8°C for up to 24 hours.

Set up and run the real-time PCR instrument

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

Note: The instrument must be configured with the block appropriate for the plate type.

1. Select the **Fast** cycling mode.

IMPORTANT! The cycling mode depends on the master mix that is used in the reaction. The cycling mode does not depend on the plate format.

2. Set up the thermal protocol.

Step	Temperature	Time	Number of cycles
UNG incubation ^[1]	25°C	2 minutes	1
Reverse transcription	50°C	15 minutes	1
Activation	95°C	2 minutes ^[2]	1
Denaturation	95°C	3 seconds	40
Anneal/Extension	60°C	1 minute	

^[1] Heat-labile UNG is completely inactivated during the initial ramp to 95°C.

^[2] Required for RT inactivation, initial denaturation, and activation of the DNA polymerase.

3. Set the reaction volume.

- 20 µL
- 10 µL

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

5. Start the run.

Perform RT-PCR

Use the RT-PCR procedure for amplification of cDNA targets.

Prepare the PCR Reaction Mix

Thaw the microbial cDNA samples on ice. Resuspend the cDNA samples by inverting the tube, then gently vortexing.

1. Gently mix the bottle of TaqMan™ Fast Advanced Master Mix, No UNG.
2. Combine the following components for the number of required reactions, plus 10% overage.

IMPORTANT! For optimal results, prepare the reaction plate on ice.

Component	Volume per well	
	96-well, 0.2-mL plates	384-well plates or 96-well 0.1-mL plates
TaqMan™ Fast Advanced Master Mix, No UNG Master Mix (2X)	10 µL	5 µL
Assay (20X)	1 µL	0.5 µL
Nuclease-free water ^[1]	7 µL	3.5 µL
Total PCR Reaction Mix volume	18 µL	9 µL

^[1] Adjust the volume of nuclease-free water for a larger volume of cDNA.

3. Vortex the PCR Reaction Mix, then centrifuge briefly.
4. Combine the following components in each well of an optical reaction plate.

Component	Volume per well	
	96-well, 0.2-mL plates	384-well plates or 96-well 0.1-mL plates
PCR Reaction Mix (from step 2)	18.0 µL	9.0 µL
Extracted microbial nucleic acid sample or elution buffer for NTC	2.0 µL	1.0 µL
Total volume	20.0 µL	10.0 µL

Note: Adjust the volume of nuclease-free water in the PCR Reaction Mix for a larger volume of cDNA.

5. Seal the plate with a MicroAmp™ Optical Adhesive Film, then vortex briefly to mix the contents.

- Centrifuge the plate briefly to collect the contents at the bottom of the wells.

IMPORTANT! Vortexing and centrifuging are required for proper mixing of the reaction components.

Set up and run the real-time PCR instrument

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

Note: The instrument must be configured with the block appropriate for the plate type.

- Select the **Fast** cycling mode.

IMPORTANT! The cycling mode depends on the master mix that is used in the reaction. The cycling mode does not depend on the plate format.

- Set up the thermal protocol for your instrument.

Step	Temperature	Time	Cycles
Enzyme activation	95°C	20 seconds ^[1]	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

^[1] Enzyme activation can be up to 2 minutes. The time should not cause different results.

- Set the reaction volume.

- 20 μ L
- 10 μ L

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

- Start the run.

Analyze the results

For more information about analyzing the results, see the appropriate resources that are listed in “Related documentation” on page 4.

Use the absolute or relative quantification ($\Delta\Delta C_t$) methods (without target normalization) to analyze results.

The general guidelines for analysis include:

- View the amplification plot; then, if needed:
 - Adjust the baseline and threshold values.
 - Remove outliers from the analysis.
- In the well table or results table, view the C_t values for each well and for each replicate group, if applicable.

Related documentation

Document	Publication Number
<i>TaqMan™ Gene Expression Assays User Guide—single-tube assays</i>	4333458
<i>TaqMan™ Gene Expression Assays Quick Reference—single-tube assays</i>	4401212
<i>Isolation of Nucleic Acid for Respiratory Tract Microbiota Profiling Experiments Quick Reference</i>	MAN0018526
<i>TaqMan™ Microbial Assays—single tube Quick Reference</i>	MAN0018536
<i>Isolation of DNA for Vaginal Microbiota Profiling Experiments Quick Reference</i>	MAN0015935
<i>DNA isolation for Urinary Tract Microbiota Profiling Experiments Quick Reference</i>	MAN0017751

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

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

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Revision	Date	Description
A.0	2 July 2021	New document for the TaqMan™ Microbe Detection and Antibiotic Resistance Assays.

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