TaqMan® Microbial Assays—single tube

Catalog Numbers A39420, A41332, A41333

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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. See "Related documentation" on page 2 for resources that contain detailed instructions and troubleshooting.

Procedural guidelines

Guidelines for nucleic acid isolation

- For detailed instructions about nucleic acid isolation for respiratory tract microbiota experiments using the MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit, see the appropriate resources that are listed in "Related documentation" on page 2.
- 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 1-Step RT-PCR

- Use TaqPath™ 1-Step RT-qPCR Master Mix, CG (Cat. No. A15299).
- 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

Prepare 1-Step RT-PCR reactions

Thaw the respiratory tract nucleic acid 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 for 1 reaction	
	Standard 96–well or 48–well Plates	384–well Plate or 96–well Fast Plate
TaqPath™ 1-Step RT-qPCR Master Mix, CG (4X)	5.0 µL	2.5 µL
TaqMan® Microbial 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	
	Standard 96–well or 48–well Plates	384–well Plate or 96–well Fast Plate
PCR Reaction Mix (see step 2)	10.0 µL	5.0 µL
Extracted respiratory tract nucleic acid sample *or* elution buffer for NTC	10.0 µL	5.0 µL
Total volume	**20.0 µL**	**10.0 µL**

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! 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 a Standard or a Fast plate format.

2. Set up the thermal protocol for your instrument.

   Table 1 Thermal protocol for TaqPath™ 1-Step RT-qPCR Master Mix, CG (StepOne™, StepOnePlus™, ViiA™ 7, 7500 Real-Time PCR System, 7500 Fast Real-Time PCR System and QuantStudio™ systems with fast cycling mode)

Stage	Step	Temperature	Time
Hold	UNG incubation[1]	25°C	2 minutes
Hold	Reverse transcription	50°C	15 minutes
Hold	Activation	95°C	2 minutes[2]
Cycling (40 cycles)	Denaturation	95°C	3 seconds
	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 appropriate for the reaction plate.
   - **96-well Standard (0.2-mL) Plate**: 20 µL
   - **96-well Fast (0.1-mL) Plate and 384–well Plate**: 10 µL

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

5. 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 2.

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
Respiratory Tract Microbiota Profiling Experiments using OpenArray™ Application Guide	MAN0017952
Respiratory Tract Microbiota Profiling Experiments using TaqMan® Array Cards Application Guide	MAN0017951
TaqMan® Gene Expression Assays User Guide—single-tube assays	4333458
Isolation of Nucleic Acid for Respiratory Tract Microbiota Profiling Experiments Quick Reference	MAN00018526

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

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

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Revision	Date	Description
A.0	18 June 2019	New document.