Respiratory Tract Microbiota Profiling Experiments v2 using TaqMan™ Array Cards

Pub. No. MAN0019508 Rev. A.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Respiratory Tract Microbiota Profiling Experiments v2 using TaqMan™ Array Cards Application Guide* (Pub. No. MAN0019507). 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.

Perform preamplification and dilute samples

Perform preamplification

Note: Preamplification of the TrueMark™ Respiratory Panel 2.0 Amplification Control is not recommended.

1. Prepare PreAmp Reaction Mix: Combine the following components for the number of required reactions plus 10% overage, then mix thoroughly by pipetting up and down.

Component	Volume per reaction
TaqPath™ 1-Step RT-qPCR Master Mix, CG	2.5 µL
TrueMark™ Respiratory Panel 2.0 PreAmp Primers[1]	2.5 µL

[1] The TrueMark™ Respiratory Panel 2.0 PreAmp Primers contains primers for all respiratory tract microbiota assays plus the TaqMan™ Universal RNA Spike In/Reverse Transcription (Xeno) Control and the RNase P RPPH1 gene, but does not contain primers for the TaqMan™ Universal Extraction Control Organism (*B. atrophaeus*).

2. Distribute PreAmp Reaction Mix, then Nuclease-free Water or sample nucleic acid to the appropriate wells of a 96-well plate.

Component	Sample reaction	No-template control (NTC) reaction
PreAmp Reaction Mix	5 µL	5 µL
Sample DNA or NEC	5 µL	—
Nuclease-free Water	—	5 µL
Total volume per reaction	**10 µL**	**10 µL**

3. Seal the plate with adhesive film.

4. Gently vortex the plate for 10 seconds to mix, then briefly centrifuge to bring contents to the bottom of the wells.

5. Place the plate in a thermal cycler that is programmed with the following thermal cycling conditions, then start the run.

Stage	Step	Temperature	Time
Hold	UNG incubation[1]	25°C	2 minutes
Hold	Reverse transcription	50°C	30 minutes
Hold	Activation	95°C	2 minutes
Cycling (14 cycles)	Denaturation	95°C	15 seconds
	Annealing/ Extension	60°C	2 minutes
Hold	Inactivation	99.9°C	10 minutes
Hold	—	4°C	Hold

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

6. Store the plate on ice until dilution for PCR (see "Dilute the preamplified product" on page 1).

Dilute the preamplified product

To determine dilution volumes, first determine the total volume of diluted preamplified sample that is required for PCR (see "Prepare and run TaqMan™ Array Cards" on page 2). We recommend that you prepare only the volume of diluted preamplified sample that is required for your experiment. The undiluted preamplified sample can be stored at –20°C long term.

1. Vortex, then briefly centrifuge the plate that contains the completed preamplification sample reactions.

2. Remove the adhesive film from the plate.

3. Prepare a 1:20 dilution of the preamplified samples in a new 96-well plate.
   a. Transfer the desired volume of the preamplified samples to a new 96-well plate (for example, 2 µL).
   b. Add the appropriate volume of Nuclease-free Water to each sample and control well (for example, 38 µL).

4. Seal the plate with new adhesive film.

5. Vortex the plate for 10 seconds, then briefly centrifuge.

6. Proceed directly to PCR (see "Prepare and run TaqMan™ Array Cards" on page 2).

Seal the plate that contains the unused portion of the undiluted preamplified product, then store at –20°C.

Prepare and run TaqMan™ Array Cards

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 TaqMan™ Fast Advanced Master Mix, No UNG.

3. For each port, combine the following components.

   (*Optional*) Use 10 µL of TrueMark™ Respiratory Panel 2.0 Amplification Control plus 10 µL of Nuclease-free Water instead of diluted preamplified product, as a positive amplification control sample.

Component	Volume per port
Diluted preamplified product	20 µL
TaqMan™ Fast Advanced Master Mix, No UNG	50 µL
Nuclease-free Water	30 µL
Total volume per port	**100 µL**

4. Fill each port with 100 µL of prepared mix.

5. Centrifuge the card at 1,200 rpm (301 × *g*) for 1 minute.

6. Repeat step 5.

7. Seal the card using TaqMan™ Array Card Sealer.

8. 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

9. Run the program.

Related documentation

Document	Pub. No.
Respiratory Tract Microbiota Profiling Experiments v2 using TaqMan™ Array Cards Application Guide	MAN0019507
TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card Product Information Sheet	MAN0019511
TrueMark™ Respiratory Panel 2.0 Amplification Control Product Information Sheet	MAN0019505

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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	5 January 2021	New document.

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