TaqPath BactoPure Microbial Detection Master Mix

Microbial detection

This quick start guide is a benchtop reference for preparing and running qPCR reactions with Applied Biosystems™ TaqPath™ BactoPure™ Microbial Detection Master Mix. Refer to the user guide for detailed product information and protocols (Pub. No. MAN0025689).

Materials

- Invitrogen™ DNA*Zap*™ PCR DNA Degradation Solutions
- Double-stranded DNase kit with 10X dsDNA buffer
- RT-PCR grade water
- Real-time PCR assays (primers and probes)
- Applied Biosystems™ TaqPath™ BactoPure™ Microbial Detection Master Mix
- Extended autoclaved TE buffer (DNA buffer)
- Applied Biosystems™ MicroAmp™ Optical Reaction Plate (or equivalent)
- Applied Biosystems™ MicroAmp™ Optical Adhesive Film and Applicator (or equivalent)
- Applied Biosystems™ QuantStudio™ Real-Time PCR System

Tips to help prevent contamination

Always follow best practices to avoid contaminating your reagents, samples, or equipment.

- Maintain separate, dedicated work areas, equipment, and supplies for setup versus amplification and analysis of products
- Set up qPCR reactions in a decontaminated laminar flow PCR hood
- Dispense sample collection (e.g., PBS) and DNA extraction (e.g., TE buffer) reagents into screw cap microcentrifuge tubes, and sterilize them in an extended autoclave cycle (80 minutes at 121°C and ~15 psi)
- Never uncap samples or reagents outside of a laminar flow hood and use sterile technique when handling open containers
- Always use DNA*Zap* solution to clean gloves, work surfaces, equipment, reagent tubes, and assay tubes before working in the hood
- Use extended autoclaved DNA buffer for sample dilutions and no-template controls (NTC)
- Change pipette tips after each dispensing cycle

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Prepare assays (optional)

Note: Assay preparation steps are recommended only for pan assays (i.e., pan-bacterial, pan-fungal, etc.).

1. In a 2 mL tube, combine the components shown in the table in the order listed. Pipet the dsDNase slowly.
2. Gently invert the capped tubes 10 times to mix. Do not vortex.
3. Gently spin down, then incubate the tubes at 40°C for 1 hour.
4. Vortex the tubes at the maximum speed for 30 seconds, making sure the mixtures move vigorously. Store at 2–8°C until ready for use, or at –20°C for long-term storage.

Component	Final concentration	Volume per 500 µL of untreated assay*
Untreated assay	20X**	500 µL
dsDNAse Final concentration	0.03 U/µL	7.5 µL
10X dsDNase buffer	1X	150 µL
RT-PCR grade water	–	Variable†

* Other volumes of assay can be treated. Do not fill the tube more than approximately 1/3 of its capacity.
** Other final concentrations of the assay can be prepared.
† Depends on the initial concentration of the untreated assay.

Prepare qPCR optical reaction plates

5. Mix the master mix well, then combine the components shown in the table in the order listed.
6. Apply the optical adhesive film to seal the plate.
7. Turn the plate upside down, then shake the plate vigorously three times.
8. Turn the plate upright, then shake the plate vigorously three more times.
9. Repeat steps 7 and 8 two more times.
10. Spin down the plate at ≥1,500 × *g* for 1 minute.

Component	Final concentration	Volume per reaction	Volume per reaction with 10% overage‡
TaqPath BactoPure Microbial Detection Master Mix (2X)	1X	5–25 µL	5.5–27.5 µL
Decontaminated assay (20X)§	1X	0.50–2.5 µL	0.55–2.75 µL
Template	>2 copies/µL	Variable	Variable
RT-PCR grade water	—	Fill to 10–50 µL	Fill to 11–55 µL
Total	**—**	**10–50 µL**	**11–55 µL**

‡ After calculating the number of reactions required, prepare qPCR mix for the appropriate number of reactions and scale those components by 10% for overage. We recommend preparing at least two replicates for each sample, including negative and positive controls.

§ If the starting concentration of your decontaminated assay is not 20X, adjust the volume of assay and water as needed to achieve a final concentration of 1X.

Run the qPCR plate

11. Set up your instrument using the provided thermal protocol.
12. Set the passive reference dye as **ROX** or **None**, depending on your version of TaqPath BactoPure master mix.
13. Load the plate in the instrument, then start the run.

Step	Temperature	Time	Cycles
Pre-read	60°C	30 seconds	Hold
Initial denature/ enzyme activation	95°C	2 minutes	
Denature	95°C	10 seconds	40
Anneal/extend	60°C	30 seconds	
Post-read	60°C	30 seconds	Hold

Learn more at **thermofisher.com/qPCR/bactopure**