qPCR microbial detection

Advancing biopharma QC testing with TaqPath BactoPure Microbial Detection Master Mix

Overview

We effectively demonstrate that TaqPath BactoPure Microbial Detection Master Mix is ideally suited for applications in biopharmaceutical manufacturing. TaqPath BactoPure Microbial Detection Master Mix enables consistent and sensitive detection of relevant targets in biopharmaceutical cell samples. Performance highlights include:

- Down to single copy detection of nucleic acid targets, even in the presence of PCR inhibitors commonly found in cell cultures
- Ability to accurately quantify both low and high copy numbers of microbial DNA
- Consistent performance across different lots helps ensure reliable results over the span of testing needs

Using qPCR for low-level contaminant detection in biopharma quality control testing

During both the pre-approval and post-approval drug manufacturing process, any contamination in the active pharmaceutical ingredient (API) or excipients in a biologic drug can cause life-threatening health hazards. Beyond its implications for patient safety, failure to quickly detect and contain contamination can impact downstream phases of the therapeutic development pipeline, resulting in high recovery costs and delays to market. Therefore, developing and implementing a comprehensive testing program for detecting contaminants is a core pillar of biologics quality control (QC).

Cell culture systems used to produce biologics like vaccines, monoclonal antibodies (mAbs), or cell and gene therapeutics are particularly susceptible to various sources of contamination, including adventitious microbial agents and mycoplasma. Contamination can occur across bioproduction stages and can be introduced via raw materials, host cells, personnel, or the manufacturing environment. Therefore, establishing routine testing with a reliable and sensitive molecular detection method for multiple contaminant targets is essential. This is particularly important for biologic drugs that must be made using aseptic manufacturing and sterile fill-finish procedures because terminal sterilization approaches may damage the final drug product.

The use of real-time quantitative PCR (qPCR) for contaminant testing is a versatile, reliable, and sensitive solution that can reduce time, effort, and cost when compared to other testing methods [1]. qPCR is integral to biopharmaceutical QC programs with established applications in adventitious agent testing, residual DNA testing, purification testing, titer testing, and batch release testing. However, qPCR testing is only as sensitive and reliable as the tools used, and qPCR master mixes are critical for generating high-quality data. Biopharmaceutical manufacturers in particular require a versatile qPCR master mix that allows sensitive and reliable detection of a wide range of contaminants, even in the presence of common PCR inhibitors.

Ultra-pure TaqPath BactoPure Microbial Detection Master Mix enables detection of low-level contamination

Applied Biosystems™ TaqPath™ BactoPure™ Microbial Detection Master Mix addresses the need for an optimized master mix for biopharma applications. It allows rapid and reliable detection of low levels of target DNA, even in the presence of inhibitors. Unlike other master mixes on the market, TaqPath BactoPure Microbial Detection Master Mix is exceptionally pure with ultra-low DNA background (Figure 1). Background DNA can lead to false positives and unpredictable results—an unacceptable outcome in therapeutic development. Because TaqPath BactoPure Microbial Detection Master Mix is ultra-purified to remove background DNA during the proprietary manufacturing process, it enables the lowest level of detection for targets of interest for both biopharmaceutical and molecular diagnostic applications (**read the white paper**).

Here, we evaluate the performance of TaqPath BactoPure Microbial Detection Master Mix in real-time PCR experiments designed to test biopharmaceutical cell samples and detect various contaminants.

Materials and methods

Cell culture supernatant from Gibco™ Viral Production Cells 2.0, a clonal cell line derived from HEK293F parental cells, was used to evaluate the performance of TaqPath BactoPure Microbial Detection Master Mix with ROX™ dye under conditions that were representative of biopharmaceutical testing. For each experiment, known copy numbers of synthetic DNA were added to Thermo Scientific™ TE buffer or diluted supernatant from cultured Viral Production Cells 2.0 (VPCs 2.0 diluted 1:1,000). Refer to figure legends for specific experimental dilution information.

In each experiment, three replicates of each sample were run in a singleplex reaction queried with assays with pan-bacterial (CH16S), pan-fungal (FungiQuant), pan-mammalian (SRS11-21), and mastadenovirus targets. All qPCR reactions contained 5 µL of sample in a 20 µL total volume. A no-template control (NTC) was used as a negative control to demonstrate the absence of bacterial contamination in TaqPath BactoPure Microbial Detection Master Mix. All experiments were run on the Applied Biosystems™ QuantStudio™ 7 Pro Real-Time PCR System with a 384-well block using fast thermal cycling conditions.

For data analysis, the C_q values for samples that did not generate detectable signals were set to 40.

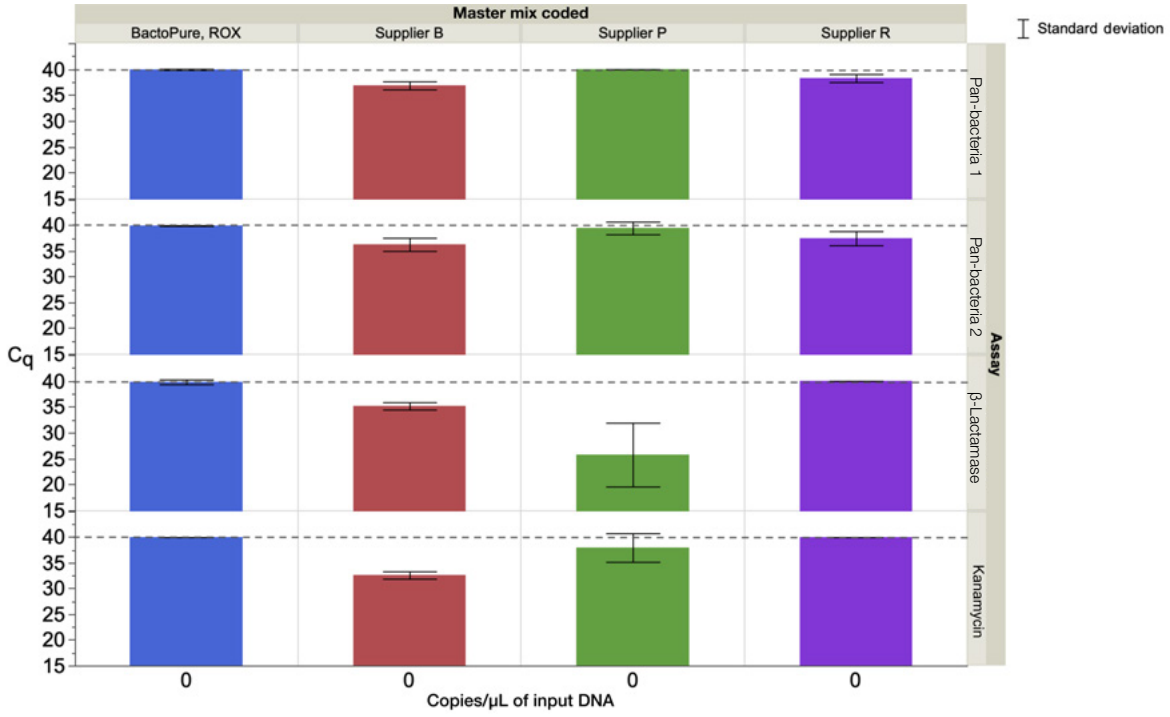

Figure 1. Background DNA is detected in other master mixes but not in TaqPath BactoPure Microbial Detection Master Mix. The amount of background level DNA in TaqPath BactoPure Microbial Detection Master Mix was compared to that of three other commercially available master mixes using assays specific to pan-bacterial targets and antibiotic resistance markers (ß-lactamase and kanamycin). TaqPath BactoPure master mix (blue) consistently reached the maximum C_q value of 40 across assays and no-template control (NTC) replicates, indicating zero amplification from background DNA. For experiment details read the **white paper**.

Results

Detection of low-concentration DNA contaminants with TaqPath BactoPure Microbial Detection Master Mix

Even low levels of contamination can pose a threat to therapeutic safety. It is thus important to reliably detect low copy numbers of microbial DNA to ensure that biopharmaceutical products do not pose a health hazard to patients, as well as to prevent delays in production. To assess the reproducibility of detection of low copy numbers of microbial DNA, we used assays that targeted various relevant contaminants.

As little as 1 copy/µL of DNA template could be detected in samples prepared with TaqPath BactoPure Microbial Detection Master Mix while generating no detectable signal for the NTC (Figure 2A). TaqPath BactoPure Microbial Detection Master Mix showed a clear dose-dependent response with increasing DNA copies per microliter. For the pan-mammalian assay, the VPCs 2.0 sample showed an insignificant difference between 0 and 1 copy/µL (Figure 2A, lower panel). This result was unsurprising and indicated the presence of mammalian DNA in the original, undiluted cell culture supernatant.

The VPCs 2.0 samples contained qPCR inhibitors. The concentration of inhibitors present in the VPCs 2.0 1:1,000 dilution was still 100 times higher than what most manufacturers would likely encounter in their samples. Low copy numbers of DNA were detected in samples prepared with TaqPath BactoPure Microbial Detection Master Mix even in the presence of abundant inhibitors.

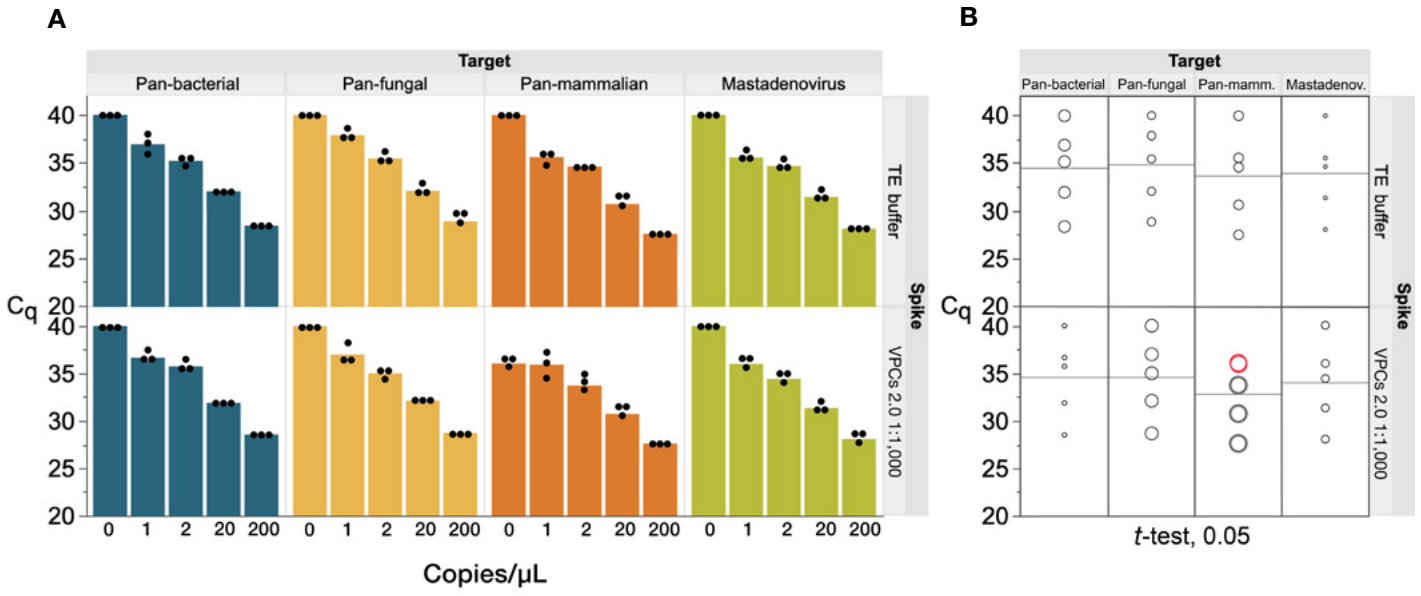

Figure 2. TaqPath BactoPure Microbial Detection Master Mix enables sensitive detection of low-level bacterial, fungal, mammalian, and viral targets in the presence of inhibitors. Known copy numbers of synthetic DNA (1, 2, 20, or 200 copies/µL) were added to TE buffer or diluted supernatant from cultured Viral Production Cells 2.0. **(A)** In both the TE buffer and VPCs 2.0 samples, TaqPath BactoPure Microbial Detection Master Mix enabled detection down to 1 copy of DNA/µL for pan-bacterial, pan-fungal, and mastadenovirus targets. **(B)** A Student's *t*-test ($P < 0.05$) confirmed significant differences between the data points in Figure 2A as indicated by the black circles. For the pan-mammalian target (Figure 2A, orange), the VPCs 2.0 sample data points showed no significant difference between 0 and 1 copy/µL. This was found by the overlapping red circles in the Student's *t*-test. This result was expected, because of trace amounts of mammalian DNA in the mammalian cell culture supernatant.

TaqPath BactoPure Microbial Detection Master Mix enables a wide dynamic range of microbial detection

The ability to accurately quantify both low and high copy numbers of microbial DNA in samples is as important as the ability to accurately detect the microbial DNA. Biomanufacturers benefit from highly precise quantification of microbial contaminants for bioburden assessments, contamination source identification, issue resolution, and other QC tests. Importantly, TaqPath BactoPure Microbial Detection Master Mix enables detection of microbial contamination over a wide dynamic range from as little as 1 copy/µL to 2 x 10^8 copies/µL (Figure 3). This enables detection of both low and high levels of contamination in samples. It is also essential to be able to quantify residual cellular DNA, a common contaminant in biologic drugs. Using pan-mammalian and mastadenovirus assays, we studied the dynamic range capability of TaqPath BactoPure Microbial Detection Master Mix at different DNA concentrations. There was little variability between replicates even for samples that contained low DNA copy numbers (Figure 3). The amplification signal was linear and proportional to DNA copy number per microliter in both TE buffer and VPCs 2.0 1:1,000 samples, as shown in Figure 3B. Taken together, these results demonstrate that the DNA can be detected over a range of eight logs in a reproducible manner regardless of the copy number with TaqPath BactoPure Microbial Detection Master Mix.

Similar results were obtained for pan-bacterial and pan-fungal targets (data not shown). Bacteria and fungi are contaminants that should be detected early in the biomanufacturing process at low copy numbers, whereas mammalian and viral targets need to be detected at both low and high copy numbers throughout bioproduction.

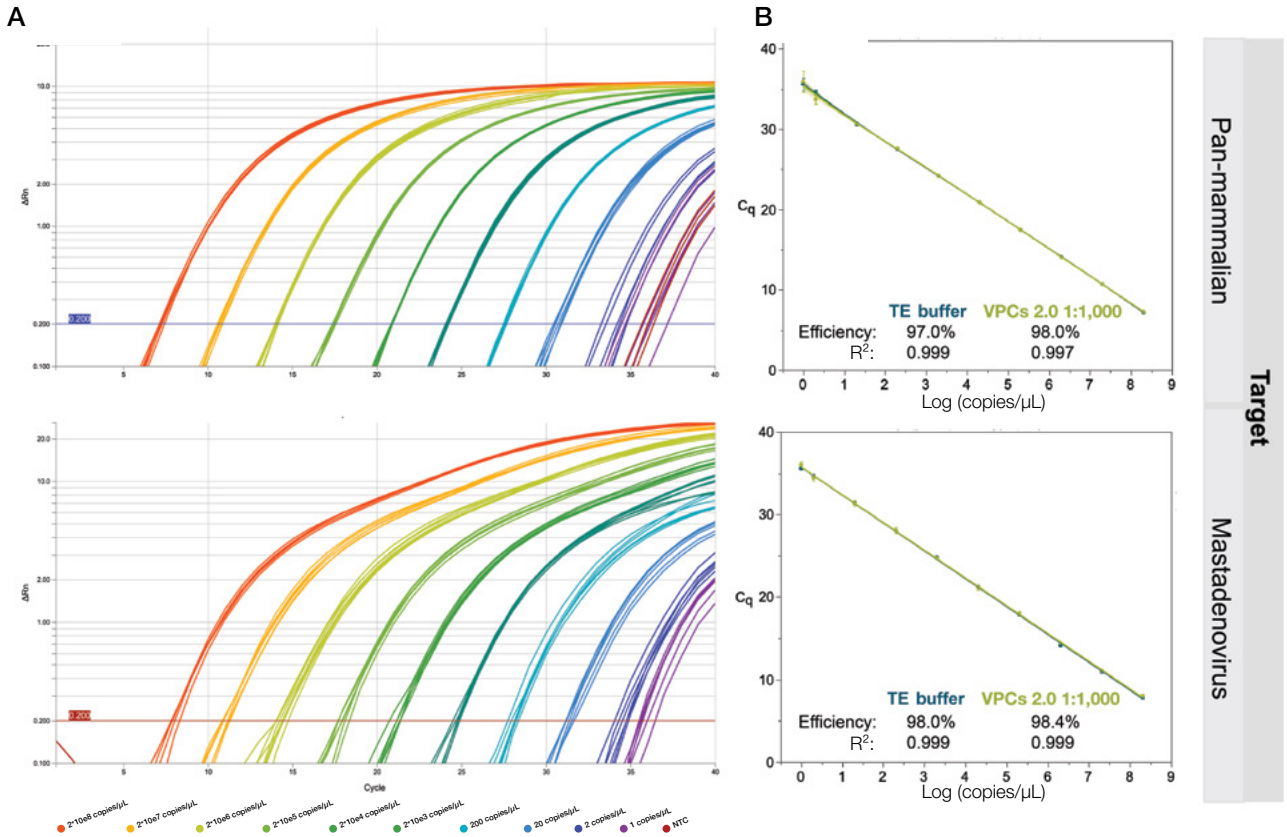

Figure 3. TaqPath BactoPure Microbial Detection Master Mix provides reliable quantification over a wide dynamic range. (A) Ten-fold synthetic DNA dilution series were created in both TE buffer and diluted Viral Production Cells 2.0 supernatant (VPCs 2.0 1:1,000 dilution). Clear, consistent amplification of pan-mammalian and mastadenovirus targets was obtained for both sample types across the dilution series. **(B)** The amplification data in linear form highlight both the efficiency and R^2 values for TE buffer and VPCs 2.0 supernatant samples. Similar results for pan-bacterial and pan-fungal targets were obtained (data not shown).

TaqPath BactoPure Microbial Detection Master Mix exhibits consistent performance across unique lots

Reliable, consistent performance across master mix manufacturing lots is increasingly important given the growing demand for pharmaceutical processing products [2]. In scenarios where biologics manufacturers cannot procure the same lot of master mix for use over the length of their product development, they rely on performance consistency to help minimize uncertainty and the need for costly repeat testing. TaqPath BactoPure Microbial Detection Master Mix has the most rigorous analytical and functional lot release QC in the industry, helping to ensure lot-to-lot consistency.

To demonstrate consistent performance, three replicates of samples containing 2 and 2 x 10^8 DNA copies/µL were run in singleplex reactions with assays specific to pan-bacterial, pan-fungal, pan-mammalian, and mastadenovirus targets. The results showed consistent performance across all lots in samples with both low and high DNA copy number (Figure 4A and Figure 4B, respectively).

Conclusion

To reduce costs due to production delays and ensure the safety of a biopharmaceutical product, it is crucial to perform stringent QC assessments that include tracking and detecting contamination from microbial and adventitious sources throughout the biomanufacturing lifecycle.

qPCR testing at multiple steps can help with timely detection of contamination, thereby preventing its spread in both upstream and downstream processing steps. TaqPath BactoPure Microbial Detection Master Mix was developed to address the need for an optimized solution that rapidly and reliably enables detection of low levels of microbial and adventitious contamination at each step of the biopharmaceutical manufacturing process.

We evaluated the performance of TaqPath BactoPure Microbial Detection Master Mix by studying contaminant DNA detection with common biopharmaceutical samples. Our results show that TaqPath BactoPure Microbial Detection Master Mix:

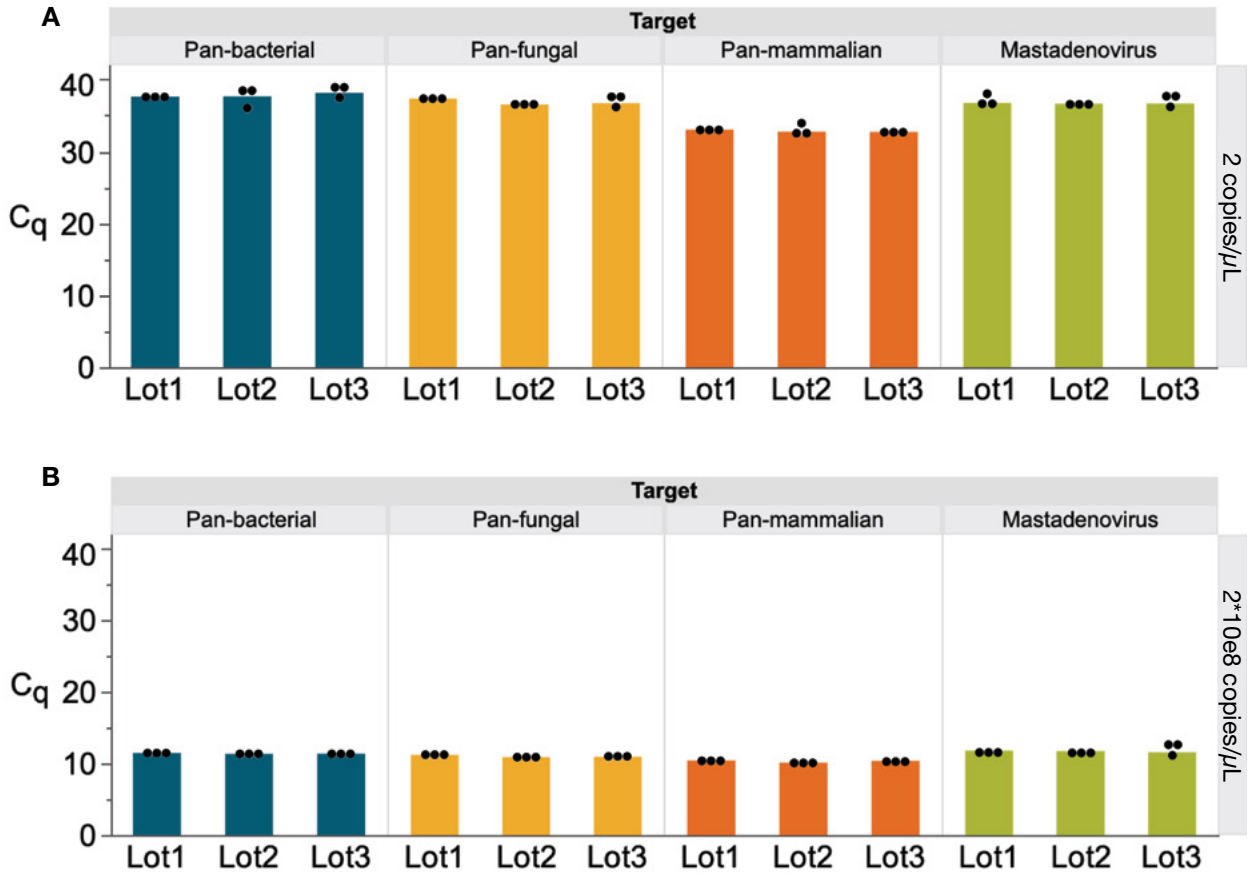

Figure 4. TaqPath BactoPure Microbial Detection Master Mix demonstrates consistent performance across lots. The consistent performance of three different lots of TaqPath BactoPure Microbial Detection Master Mix was demonstrated using both 2 and 2 x 10^8 copies of DNA template per microliter and assays specific to pan-bacterial, pan-fungal, pan-mammalian, and mastadenovirus targets. For each lot, the assay panel was run with three replicates on the QuantStudio 7 Pro Real-Time PCR System. C_q concordance is observed across the three lots of master mix, at both **(A)** low and **(B)** high copy numbers.

1. Consistently enables detection of low levels of bacterial, fungal, viral, and mammalian DNA, which are the main sources of contamination for biopharmaceutical products.
2. Detects contaminant DNA over a wide dynamic range (over a span of eight logs), enabling accurate detection of both low and high levels of contamination in biopharmaceutical samples.
3. Exhibits lot-to-lot consistency, an essential feature of this qPCR testing solution that helps ensure consistency in manufacturing safe biopharmaceutical products across different batches, especially when manufacturers are unable to procure the same lot of TaqPath BactoPure Microbial Detection Master Mix.

TaqPath BactoPure Microbial Detection Master Mix has also been validated for multiplexing up to four targets in one reaction. This allows for multiple targets and controls to be run simultaneously for greater efficiency.

References

1. Barone PW, Wiebe ME, Leung JC et al. (2020) Viral contamination in biologic manufacture and implications for emerging therapies. *Nat Biotechnol* 38(5):563–572. doi:10.1038/s41587-020-0507-2.
2. Foster T, Patel P, Skiba K (2021) Four ways pharma companies can make their supply chains more resilient. McKinsey & Company. **https://www.mckinsey.com/industries/life-sciences/our-insights/four-ways-pharma-companies-can-make-their-supply-chains-more-resilient**

Product information

PCR option*	Compatible reporter dyes	Recommended master mix formulation
Singleplex (1 probe)	FAM, VIC, ABY	TaqPath BactoPure Microbial Detection Master Mix with ROX dye
Multiplex (2–3 probes)		
Multiplex (>3 probes)	JUN, FAM, VIC, ABY	TaqPath BactoPure Microbial Detection Master Mix (no ROX dye)

* For detailed information about multiplex reactions, see **Applied Biosystems™ TaqMan™ Assay Multiplex PCR Optimization Application Guide** (Pub. No. MAN0010189).

Ordering information

Product	Quantity	Cat. No.
TaqPath BactoPure Microbial Detection Master Mix with ROX dye	1 mL	A52699
	5 mL	A52700
	5 x 1 mL	A52701
	50 mL	A52702
TaqPath BactoPure Microbial Detection Master Mix (No ROX)	1 mL	A52703
	5 mL	A52704
	5 x 1 mL	A52705
	50 mL	A52706

Please note that Thermo Fisher Scientific also offers qPCR solutions for mycoplasma testing, residual DNA testing, and adventitious virus (specifically Mouse Minute Virus, Vesivirus, and Sf-Rhabdovirus) testing.

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