Microbial detection

TaqPath BactoPure Microbial Detection Master Mix supports low-level target detection for biopharmaceutical and molecular diagnostic applications

Introduction

Applied Biosystems™ TaqPath™ BactoPure™ Microbial Detection Master Mix is designed for quantitative polymerase chain reaction (qPCR) applications and optimized for rapid low-level microbial detection, even in the presence of inhibitors. The proprietary manufacturing process ensures that the TaqPath BactoPure Microbial Detection Master Mix is devoid of detectable spurious DNA that often results in background noise for other products on the market. This novel formulation allows the highly reproducible detection of ultralow-level microbial targets from a wide variety of samples for both biopharmaceutical and molecular diagnostic applications.

Molecular diagnostics

Molecular diagnostics (MDx) is a powerful tool to guide patient management in areas such as infectious diseases, inherited conditions, and cancer. Historically, confirming a viral or bacterial infection often required isolation and growth of the pathogen in appropriate culture systems, involving highly skilled personnel and a lengthy turnaround time to confirm a diagnosis. The implementation of molecular methods has revolutionized the diagnostic sector. Molecular applications, such as PCR technology, offer a fast turnaround time and are now widely used for detection, quantification, and typing of different microbial agents.

Clinical laboratories can use *in vitro* diagnostic (IVD) tests and molecular assays for patient testing. Molecular assays can cover a wide variety of methods, including PCR-based assays for the detection of microbial infections. Molecular assays are regulated depending on the territory in which they are used. In the United States, IVD tests are validated by manufacturers and regulated by the U.S. Food and Drug Administration (FDA), whereas molecular assays are developed, manufactured, and validated by an individual laboratory. In Europe, molecular assays or in-house assays (IHAs) are subject to regulation (EU) 2017/746, known as *In Vitro* Diagnostic Regulation (IVDR).

PCR-based molecular diagnostics enable fast sample-to-result times and the ability to develop tests for various microbial agents with existing laboratory infrastructure. Independent of the test, molecular assays must reliably detect their targets, even in the presence of inhibitors. It is therefore crucial for molecular assay developers to use only the highest-quality raw materials. For developers of qPCR-based molecular assays, using an exceptionally reliable master mix with a robust performance in detecting low-level targets is key to success.

Biopharmaceuticals

Stringent quality control encompassing raw material fulfillment, manufacturing processes, and final product validity is crucial throughout the biopharmaceuticals industry. Introduction of adventitious agents into the manufacturing process can lead to unforeseeable changes, potentially impacting the activity or stability of the relevant biological product. Moreover, microbial contamination of active ingredients and drug products could pose health hazards to patients. To ensure consistency, quality, and safety of the final product, contamination control throughout the entire manufacturing process—from raw material selection to lot release—is essential.

Cell culture systems are widely used to produce biopharmaceuticals, such as monoclonal antibodies, vaccines, therapeutic proteins, and cell and gene therapies. Bacteria (such as mycoplasmas), fungi, and viruses are common microbial contaminants. Mycoplasmas and viral agents are difficult to control, and monitoring cell culture performance alone may not identify all contamination. qPCR technology offers a highly specific and sensitive solution to detect contamination at various stages of biologics manufacturing, even at low copy numbers. Due to quick turnaround times, implementing qPCR testing at multiple steps during biopharmaceutical manufacturing processes can help detect contamination early, thereby preventing the spread of adventitious agents and keeping downstream work areas clean.

Whether the origin of a sample is human, animal, plant, or soil-based, many diverse types of tests are used to monitor adventitious microbial agents during process development, preclinical and clinical biologics development, and post-approval manufacturing processes. The selection of adequate testing methodology in these areas is critical for effective contamination monitoring. Using qPCR technology for this testing can have significant advantages such as qu
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In addition to its utility for adventi
BactoPure Microbial Detection M
range, making it the premier reag
and verifying DNA product specif
vaccines not only require reliable confirmation of the identity and concentration of nucleic acids in the final product but also a clear understanding of biodistribution and persistence in the host. Depending on product design, DNA integration studies may also be required.

Performance of the TaqPath BactoPure Microbial Detection Master Mix

Reproducible, sensitive detection

Figure 1. Reliable low-copy detection (pan-bacterial and pan-fungal qPCR TaqMan assay). (A) Samples containing known copy numbers of a synthetic DNA target were amplified using either a pan-bacterial or a pan-fungal qPCR assay. Quadruplicate testing was performed for all data points, using the indicated master mixes. The 40-cycle qPCR was performed on the Applied Biosystems™ QuantStudio™ Real-Time PCR System (384-well block) using fast thermal cycling. Note that the C_q value for each sample that did not result in signal was set to 40. **(B)** A Student's t-test (0.05) confirms significant differences between all the icrobial Detection Master Mix (as indicated by the black circles and the

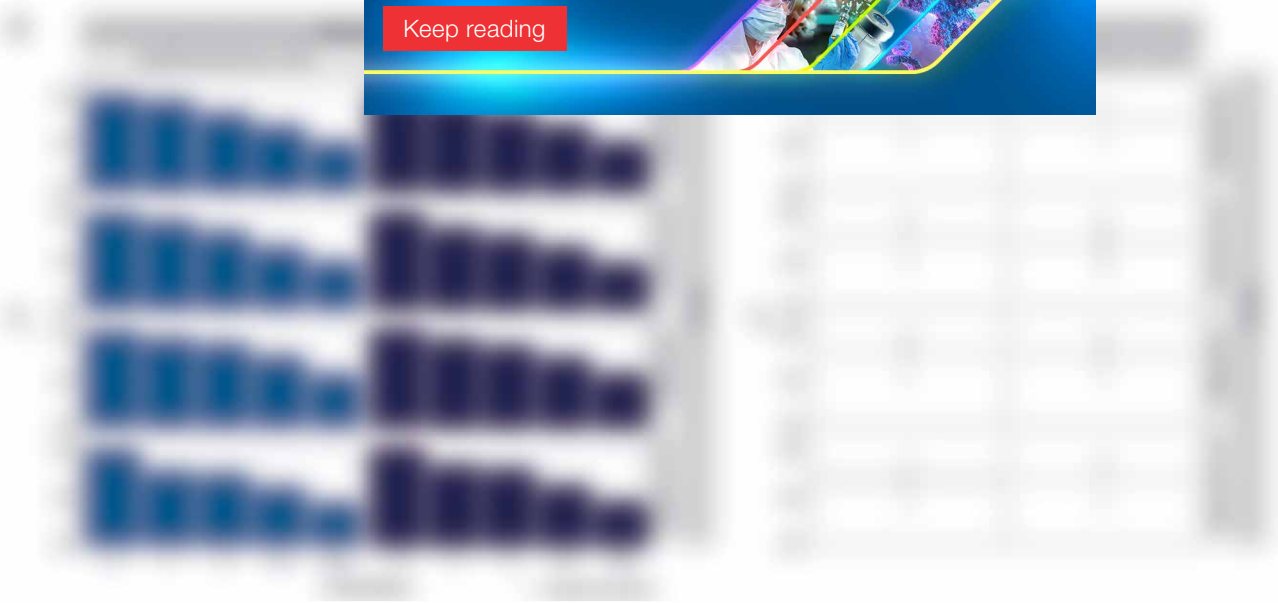

Figure 2. Reliable low-copy detection (pan-bacterial, pan-fungal, pan-mammalian, and beta-lactamase resistane gene qPCR TaqMan assays). (A) Samples containing different copies of a synthetic DNA assay target were amplified using the indicated assay. Quadruplicate testing was performed for all data points, using the TaqPath BactoPure Microbial Detection Master Mix either with or without ROX dye. The 40-cycle qPCR was performed on the CFX Opus PCR System (96-well block) from Bio-Rad. Note that the C_q value for each sample that did not result in signal was set to 40. **(B)** A Student's t-test (0.05) confirms significant differences between all the serial dilution data points for all four assays (as indicated by the black circles and their clear separation).

No background noise

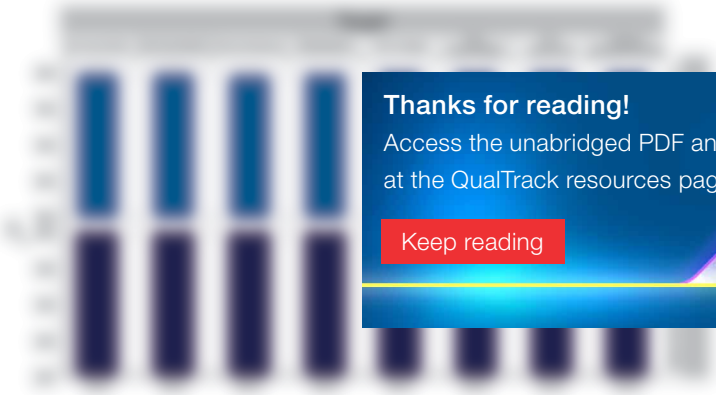

Figure 3. No background noise. NTC samples were tested with the indicated TaqMan assays on the QuantStudio Real-Time PCR System (384-well block, fast thermal cycling, 40 cycles total), using TaqPath BactoPure Microbial Detection Master Mix with or without ROX dye. Each assay tested 4 replicates. Note that the C_q value for each sample that did not result in signal was set to 40.

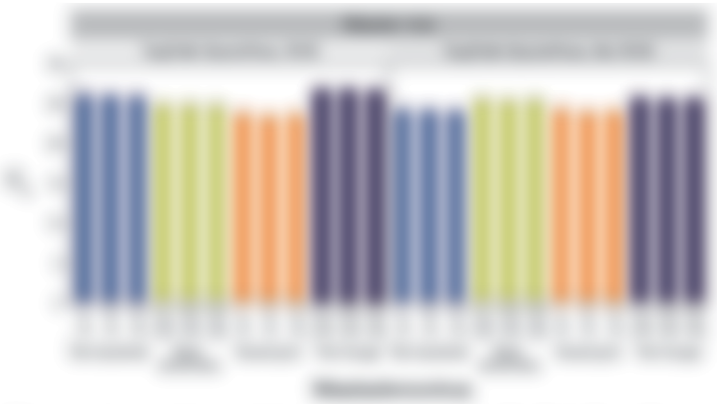

Figure 4. Lot-to-lot consistency. Three lots of the TaqPath BactoPure Microbial Detection Master Mix were tested using the indicated TaqMan assays. Samples containing 1,000 copies of a synthetic DNA target were amplified. The PCR run was performed on the QuantStudio Real-Time PCR System (384-well block, fast thermal cycling).

Dynamic range

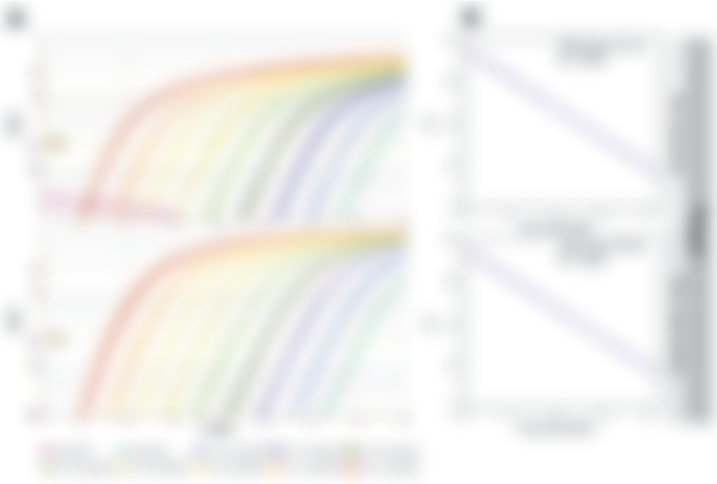

Figure 5. Excellent dynamic range of TaqPath BactoPure Microbial Detection Master Mix. (A) Amplification plots from real-time PCR for a dilution series of four replicates of DNA that were amplified using a pan-bacterial (upper panel) assay and a pan-mammalian (lower panel) assay on the QuantStudio Real-Time PCR System (fast thermal cycling). **(B)** Amplification results are expressed as mean C_q values, highlighting both efficiency and linearity (R^2).

Inhibitor tolerance

Figure 6. TaqPath BactoPure Microbial Detection Master Mix performance in the presence of inhibitors. Three inhibitors (hematin, heparin, and humic acid) were added to samples for analysis with the pan-bacterial qPCR TaqMan assay to assess the impact of these inhibitors. The tests used 1,000 copies of synthetic DNA template (200 copies/µL) and were run on the QuantStudio Real-Time PCR System [illegible] inhibitors on the TaqPath BactoPure [illegible] suppliers B and P was calculated by [illegible] the C_q values of inhibited samples [illegible] ed as ΔC_q (dC_q).

Figure 7. TaqPath BactoPure Microbial Detection Master Mix performance using crude or purified samples. The performance of the TaqPath BactoPure master mix for purified samples or crude lysates is shown for three different sample types (blood, saliva, and swab). Samples containing a synthetic DNA template were either purified via extraction with the MagMAX CORE kit (orange) or prepared as a crude lysate with the DNA Extract All Reagents Kit (blue). qPCR reactions containing ~500 copies of template DNA were run on the QuantStudio Real-Time PCR System (fast thermal cycling).

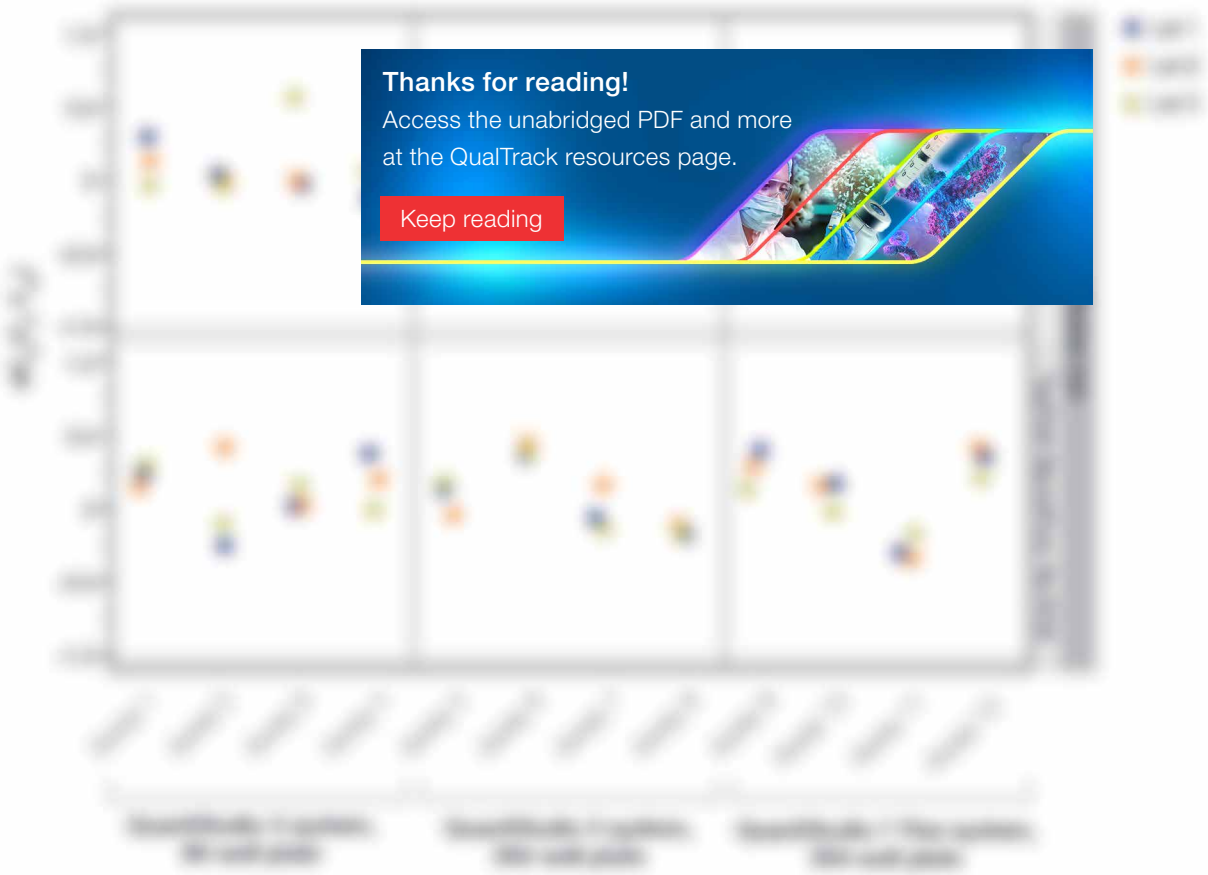

Figure 8. Benchtop stability. Three lots of the TaqPath BactoPure Microbial Detection Master Mix were tested using a variety of TaqMan assays and performed on different qPCR instruments using standard thermal cycling. The difference between the C_q values obtained 0 hr (T_0) and 24 hr (T_{24}) post-assembly of the reaction is expressed as ΔC_q (dC_q).

Optimized for multiplexing

Simultaneous amplification and detection of multiple targets in the same reaction can be beneficial for many reasons, such as the inclusion of a quality control, conservation of patient samples, or increased efficiency. The TaqPath BactoPure Microbial Detection Master Mix is optimized for multiplexing applications, allowing additional exogenous or endogenous controls or targets to be run simultaneously. The master mix containing ROX dye supports up to triplex reactions, and the formulation without ROX dye supports up to quadruplex reactions.

The performance of the TaqPath BactoPure Microbial Detection Master Mix (with ROX dye) to amplify the enterotoxigenic *E. coli wzy* gene alone (singleplex) or while amplifying two additional targets (the *stn1* and *stx2* genes) in the same reaction (triplex) is shown in Figure 9A. For the TaqPath BactoPure Microbial Detection Master Mix (no ROX dye), the performance of amplifying one target (*Salmonella* spp.) was compared to amplifying the same target in a quadruplex reaction also amplifying the *wzy*, *stn1*, and *stx2* genes (Figure 9B).

The TaqPath BactoPure master mix maintains comparable performance between singleplex and multiplex reactions, as demonstrated by the linear amplification plots (R^2) and qPCR efficiencies.

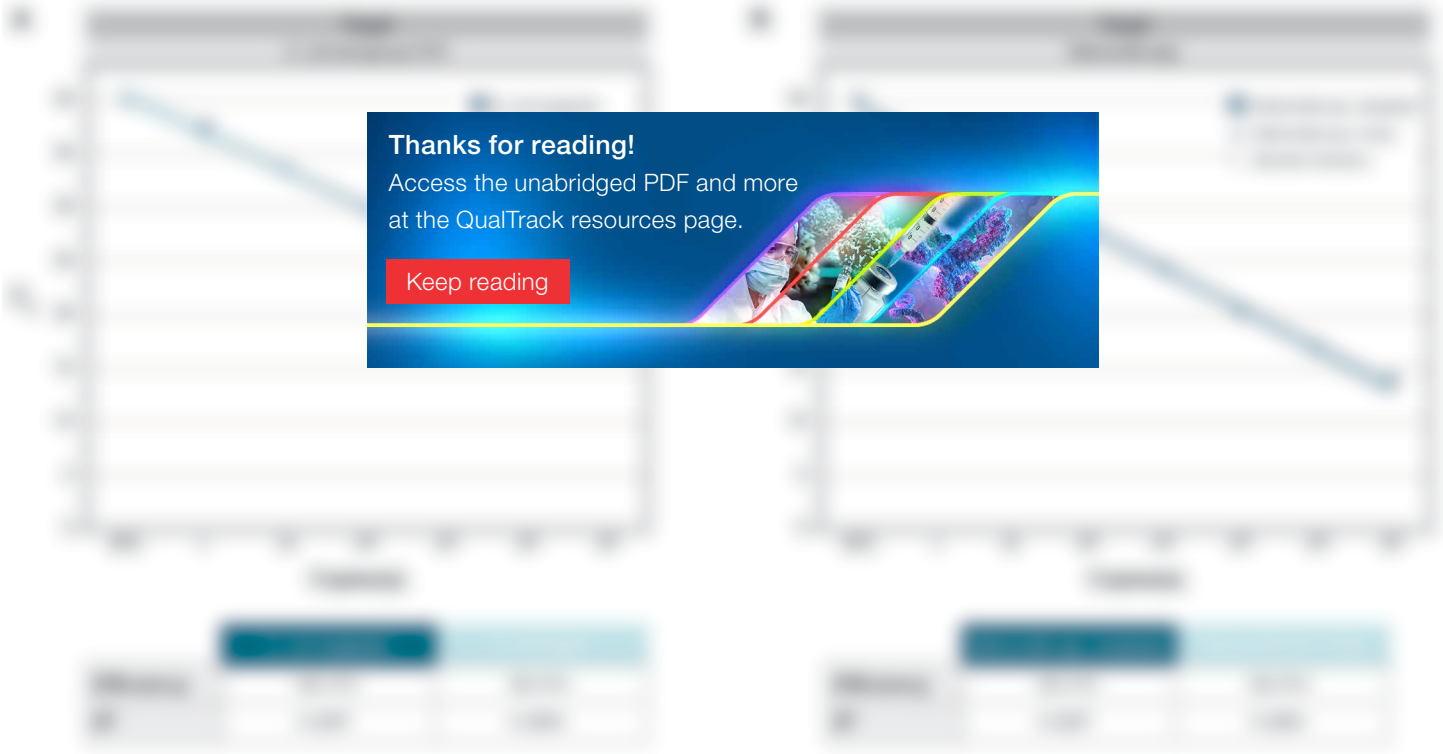

Figure 9. TaqPath BactoPure Microbial Detection Master Mix is optimized for multiplexing. The performance of the TaqPath BactoPure master mix in multiplex reactions was assessed. For each target, a 10-fold DNA dilution series was prepared, and four replicates for each dilution point were run on the QuantStudio Real-Time PCR System (fast thermal cycling), either as singleplex or multiplex reactions. **(A)** Amplification of the enterotoxigenic *E. coli wzy* gene in singleplex and triplex reactions using the TaqPath BactoPure Microbial Detection Master Mix (with ROX dye). **(B)** Amplification of the *Salmonella* spp. target in singleplex and quadruplex reactions using the TaqPath BactoPure Microbial Detection Master Mix (no ROX dye).

Excellent manufacturing consistency

We understand the importance of our product's performance reliability to your test quality and data interpretation needs. TaqPath BactoPure Microbial Detection Master Mix is manufactured in an ISO 13485–certified and FDA-registered facility that adheres to cGMP principles to ensure excellent manufacturing consistency.

We perform an extensive set of quality control analytical methods and functional testing on each lot of TaqPath BactoPure Microbial Detection Master Mix to ensure the highest level of performance and lot-to-lot consistency. In addition to testing the efficiency of qPCR reactions by standard curve analysis in both singleplex and multiplex formats, our functional testing includes an exhaustive panel of contamination assays to confirm the master mix is free of detectable spurious DNA. Both positive and NTC samples are screened with assays targeting the following gene sets:

- Pan-bacterial
- Pan-fungal
- Pan-eukaryotic
- Pan-mammalian
- Antibiotic resistance genes (beta-lactamase, methicillin, vancomycin, and kanamycin)

A Certificate of Analysis (CoA) is available for each lot of TaqPath BactoPure Microbial Detection Master Mix that includes a description of each quality control parameter, method, acceptance criteria, and final conformity result to deliver transparency for your downstream traceability requirements.

Summary

Benefits of TaqPath BactoPure Microbial Detection Master Mix include:

- **Low-level detection**—sensitive and reliable detection of DNA from bacteria, fungi, eukaryotes, mammals, and viruses as well as antibiotic resistance markers
- **Tolerance of inhibitors**—maintains function in the presence of inhibitors typically found in biopharmaceutical, molecular diagnostic, and research applications, both from purified samples and crude lysates
- **Wide dynamic range**—up to 8 orders of magnitude of dynamic range enables accurate detection on both low- and high-concentration samples
- **Optimized for multiplexing**—available in two formulations that enable single- to four-target detection per reaction
- **Excellent benchtop stability**—retains consistent performance in preassembled reactions for at least 24 hr
- **High-throughput liquid-handling compatible**—the room temperature stability of this mix (up to 72 hr) allows users to achieve results on the last plate that parallel those on the first plate
- **Excellent manufacturing consistency**—labeled "For Laboratory Use" and manufactured in an ISO 13485–certified and FDA-registered facility, which adheres to cGMP principles
- **Long product shelf life**—guaranteed minimum shelf life of at least 1 year upon receipt (exact expiry date printed on product label and lot-specific CoA)
- **Regulatory support**—readily available compliance documents

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