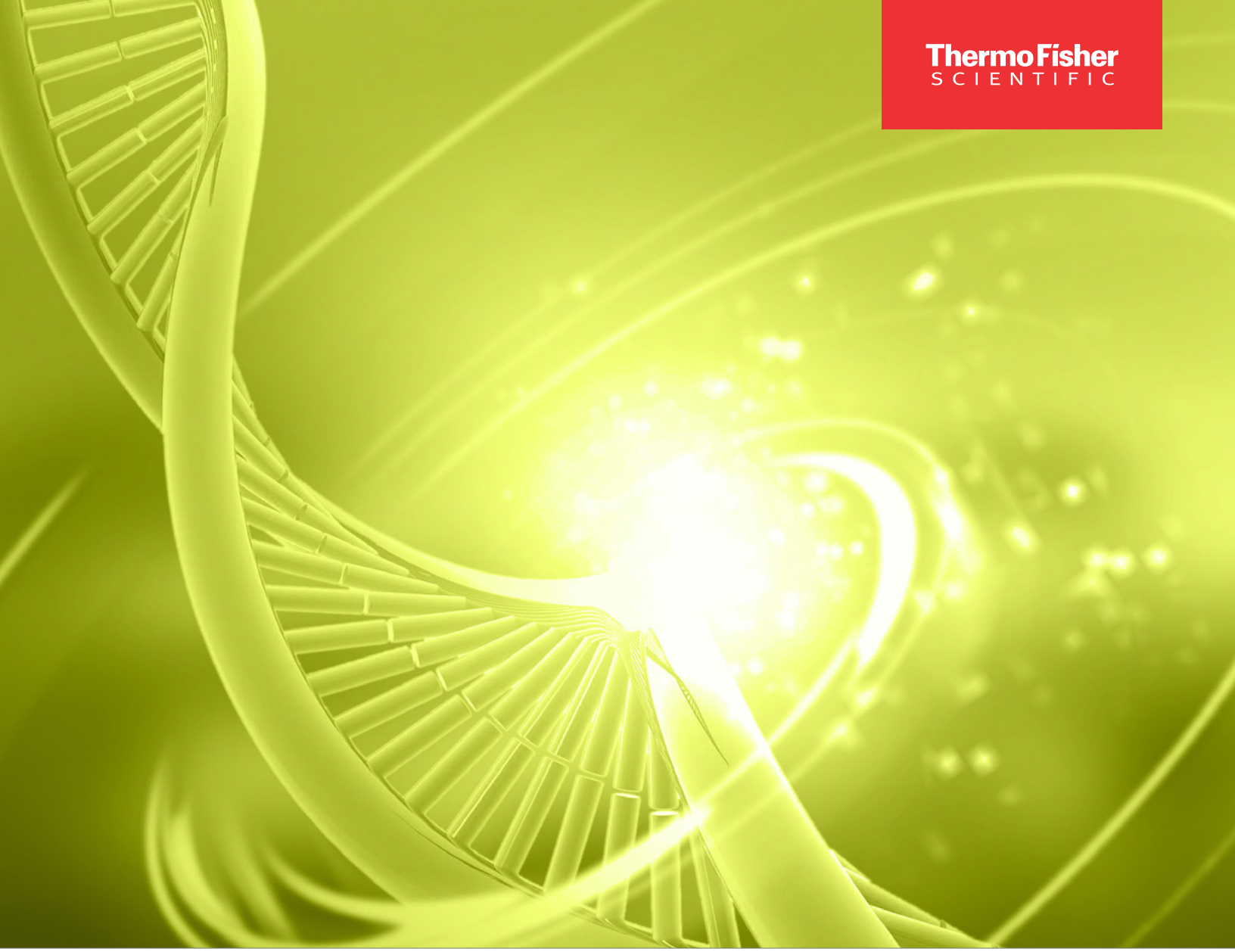


qPCR

TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient

applied biosystems

The Applied Biosystems™ TaqMan™ 2.5X Lyo-Ready qPCR Master Mix With Excipient is designed to be incorporated directly into a lyophilization process. No additional excipients are required—just add your primers and probes and proceed to the lyophilization. Lyophilization guidelines are available to help customers reduce the amount of time needed to optimize the lyophilization process and fast-track conversion from the liquid product to the lyophilized material while retaining the quality of qPCR results.

This reagent is manufactured according to ISO 13485 requirements. All lots are functionally tested to support lot-to-lot reproducibility. It is well suited for multiplexing and challenging samples, and can be used to reliably discriminate between low copy numbers of nucleic acids present in the sample.

Benefits of TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient

- **Ready to use in lyophilization**—lyophilization guidelines with a comprehensive troubleshooting section are available (please refer to Pub. No. MAN0028542)
- **Optimized for multiplexing**—up to 5 targets per reaction can be detected
- **Tolerant of PCR inhibitors**—compatible with purified samples and crude lysates used in biopharmaceutical, molecular diagnostic, and research applications
- **Lot-to-lot consistency**—highly reproducible detection

Ready to use in lyophilization

TaqMan 2.5X Lyo-Ready 1-Step qPCR Master Mix With Excipient has been optimized for consistent performance before and after lyophilization (Figure 1). Comprehensive lyophilization guidelines are available to help shorten your path from liquid to lyophilized product. The excipient's proprietary formulation ensures fast rehydration of the lyophilized assay after a liquid sample is added.

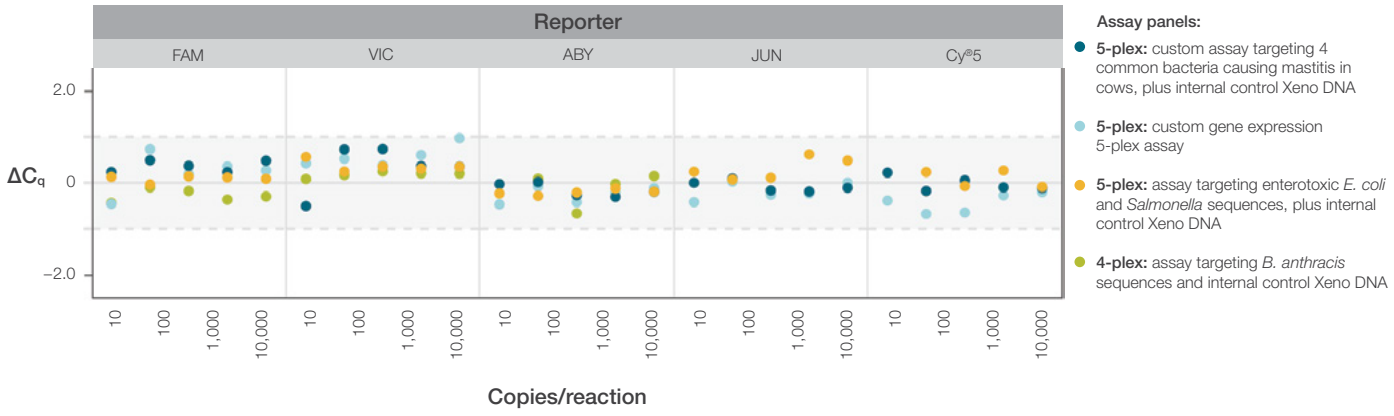
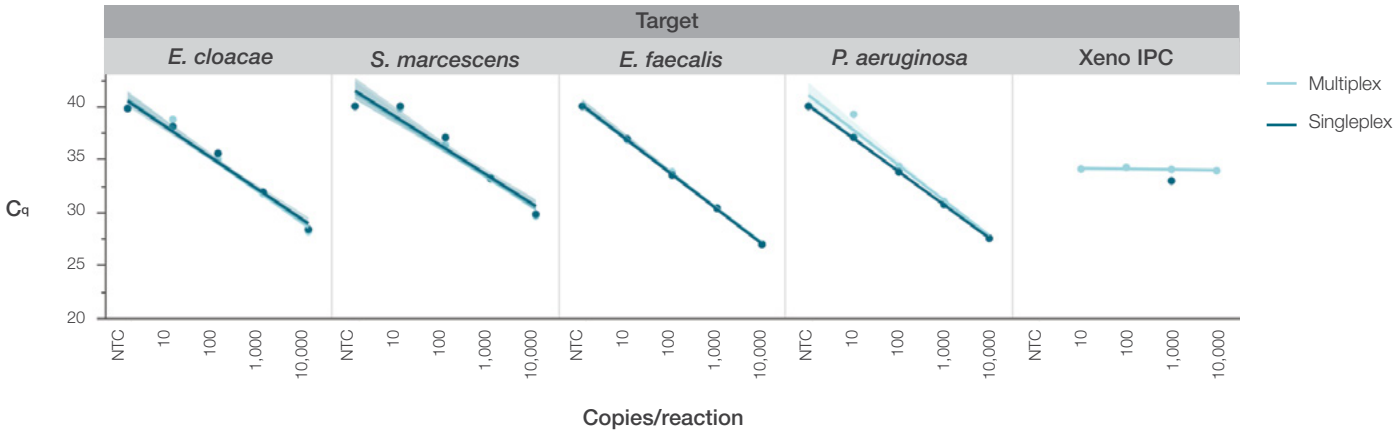


Figure 1. Optimized for lyophilization (performance before and after lyophilization). The performance of the TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient, before and after lyophilization, was evaluated with synthetic templates and an extended panel of Applied Biosystems™ TaqMan™ multiplex assays. For each of the assays, three replicates of a 10-fold dilution series of DNA were quantified using pre-lyophilized (liquid) and lyophilized master mix. To show performance changes, the ΔC_q for each sample was determined as the difference between the C_q values of the lyophilized master mix and the pre-lyophilized master mix. As demonstrated in the plot, TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient has ΔC_q values between 1 and –1 across all targets and dilutions, indicating comparable performance between both states of the master mix.

Optimized for multiplexing

TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient has been validated for multiplexing with up to 5 targets per reaction, allowing additional assays and controls to be run simultaneously for increased efficiency. Figure 2 illustrates this multiplexing capability by comparing performance between singleplex and multiplex reactions.



	E. cloacae		*S. marcescens*		*E. faecalis*		*P. aeruginosa*		Xeno IPC
Plex	**Efficiency**	**R^2**	**Efficiency**	**R^2**	**Efficiency**	**R^2**	**Efficiency**	**R^2**	**Slope**
1-plex	100.5%	0.978	94.9%	0.994	100.5%	0.977	106.7%	0.993	0.00
5-plex	92.7%	0.997	98.4%	0.991	100.9%	0.975	90.6%	0.995	0.06

Figure 2. Optimized for multiplexing. The performance of TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient in multiplex assays was assessed using singleplex and 5-plex reactions. For each target, a 10-fold DNA dilution series was prepared, with four replicates of each dilution. A constant concentration of Xeno DNA, as an internal positive control (IPC), was added across all dilutions. The figure illustrates that TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient maintains comparable performance between singleplex and 5-plex reactions for all of the microbial targets, while maintaining the same C_q for the IPC, as indicated by the linear amplification plots, qPCR efficiencies, and R^2 values.

Tolerant of inhibitors

The unique proprietary formulation allows robust performance even in the presence of substances that can inhibit PCR (Figure 3), resulting in effective template detection from both purified DNA and crude lysate (Figure 4).

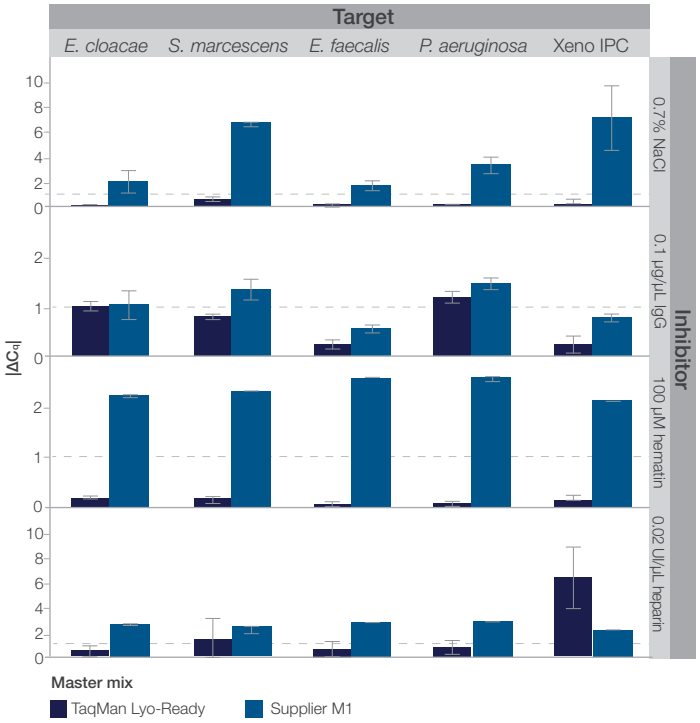


Figure 3. High inhibitor tolerance. The inhibitor tolerance of TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient was compared to another supplier's master mix. For each master mix, four replicates of samples containing 10,000 copies/reaction of artificial template were amplified using a custom 5-plex assay targeting 4 microbial targets plus Xeno DNA both with and without the following inhibitors: NaCl (0.7%), IgG (0.1 µg/µL), hematin (100 µM), or heparin (0.02 U/µL). To show performance changes in the presence of inhibitors, the absolute value of ΔC_q ($|\Delta C_q|$) for each sample was determined as $|\Delta C_q| = |C_{q\ \text{inhibited sample}} - C_{q\ \text{non-inhibited sample}}|$, with $|\Delta C_q|$ of ≤1 considered not significant (dashed gray line). As demonstrated in the plot, TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient has low $|\Delta C_q|$ values across targets, indicating comparable performance between inhibited and non-inhibited samples. The other master mix has substantially higher $|\Delta C_q|$ values for all targets, indicating it has lower inhibitor tolerances and is more susceptible to the effects of inhibitors on performance.

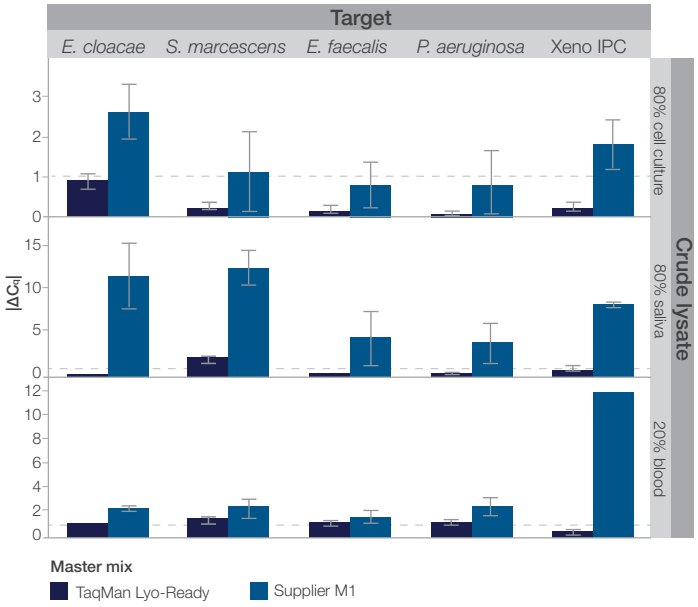


Figure 4. Consistent performance across lysates and samples. The tolerance of TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient to crude lysates of cell culture (K562 cell line), saliva, and whole blood was compared to another supplier's master mix. For each master mix, three replicates of samples containing 10,000 copies/reaction of artificial template were amplified using a custom 5-plex assay targeting 4 microbial targets plus Xeno DNA, both with and without crude lysate: 80% of the reaction volume of cell culture crude lysate, 80% for saliva, or 20% for whole blood. The crude lysates were obtained using the Invitrogen™ Cells-to-C_T™ 1-Step TaqMan™ Kit (Cat. No. A25605), TaqMan™ SARS-CoV-2 Fast PCR Combo Kit 2.0 (Cat. No. A51607), and Applied Biosystems™ DNA Extract All Reagents Kit (Cat. No. 4402616), respectively. To show performance changes in the presence of inhibitors, the absolute ΔC_q ($|\Delta C_q|$) for each sample was determined as $|\Delta C_q| = |C_{q\ \text{inhibited sample}} - C_{q\ \text{non-inhibited sample}}|$, with $|\Delta C_q|$ of ≤1 considered not significant (dashed gray line). As demonstrated in the plot, TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient has low $|\Delta C_q|$ values across targets, indicating comparable performance between inhibited and non-inhibited samples. The other master mix has substantially higher $|\Delta C_q|$ values for most targets, indicating it has lower inhibitor tolerance and is more susceptible to the effects of inhibitors on performance.

Lot-to-lot consistency

TaqMan 2.5X Lyo-Ready qPCR Master Mix has been optimized for highly reproducible detection of targets from a wide variety of samples. Consistent C_q values were observed with three different lots of TaqMan Lyo-Ready qPCR Master Mix across multiple assays (Figure 5). You can have confidence in your results and enjoy exceptional lot-to-lot consistency with TaqMan Lyo-Ready qPCR Master Mix.

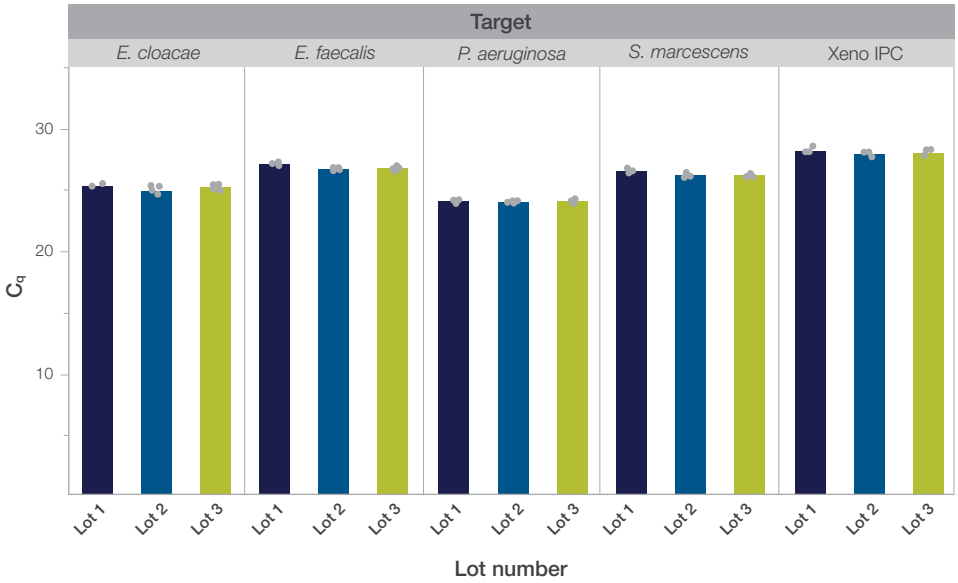


Figure 5. Exceptional lot-to-lot performance consistency. The consistent performance of four replicates of three different lots of TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient was demonstrated using 10,000 copies/reaction of DNA template and a custom 5-plex assay (targeting 4 common mastitis-causing bacteria plus Xeno DNA). Excellent C_q concordance between is seen across the lots for all targets.

Dynamic range and limit of detection (LOD)

TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient demonstrates a wide dynamic range (Figure 6) and reliable differentiation between 0 and 5 copies of DNA (Figure 7).

Reagent for further manufacturing

TaqMan Lyo-Ready master mixes are reagents used in further manufacturing (lyophilization) and are labeled “Caution: For use as a raw material in further manufacturing applications.”

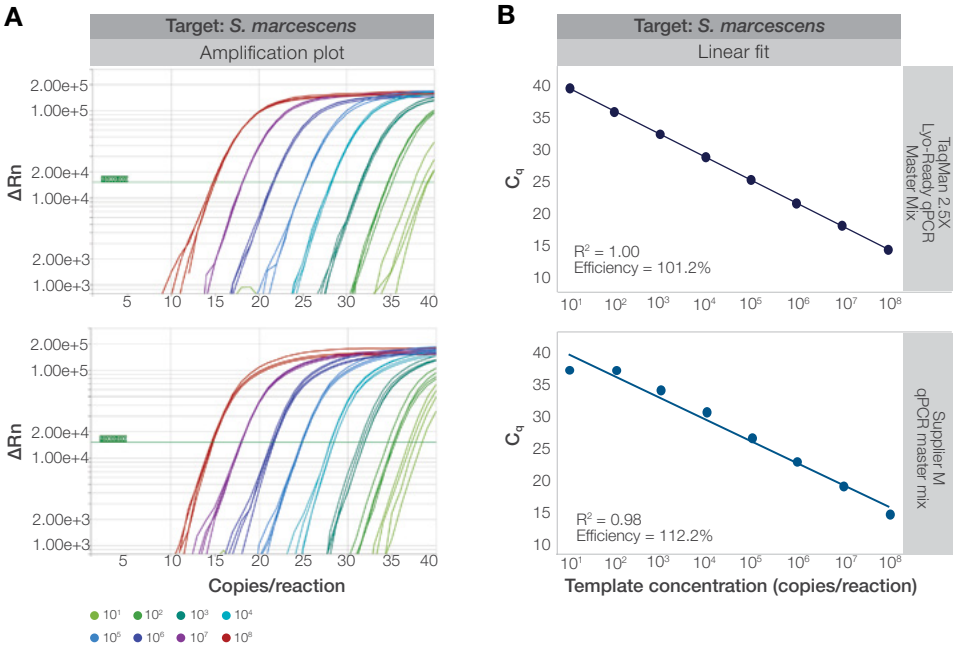


Figure 6. Linear dynamic range of 7 orders of magnitude. The linear dynamic range of TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient was compared to that of another supplier’s master mix. The experiment used four replicates of a 10-fold DNA dilution series, from 10 copies/reaction to 10^8 copies/reaction, and a custom 5-plex assay (targeting 4 common mastitis-causing bacteria plus Xeno DNA). **(A)** Amplification curves and **(B)** linearized amplification data with PCR efficiency and R^2 values are shown for the *S. marcescens* target. The results demonstrate consistent amplification across eight 10-fold dilution points for TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient, and a lower dynamic range for the other supplier’s master mix.

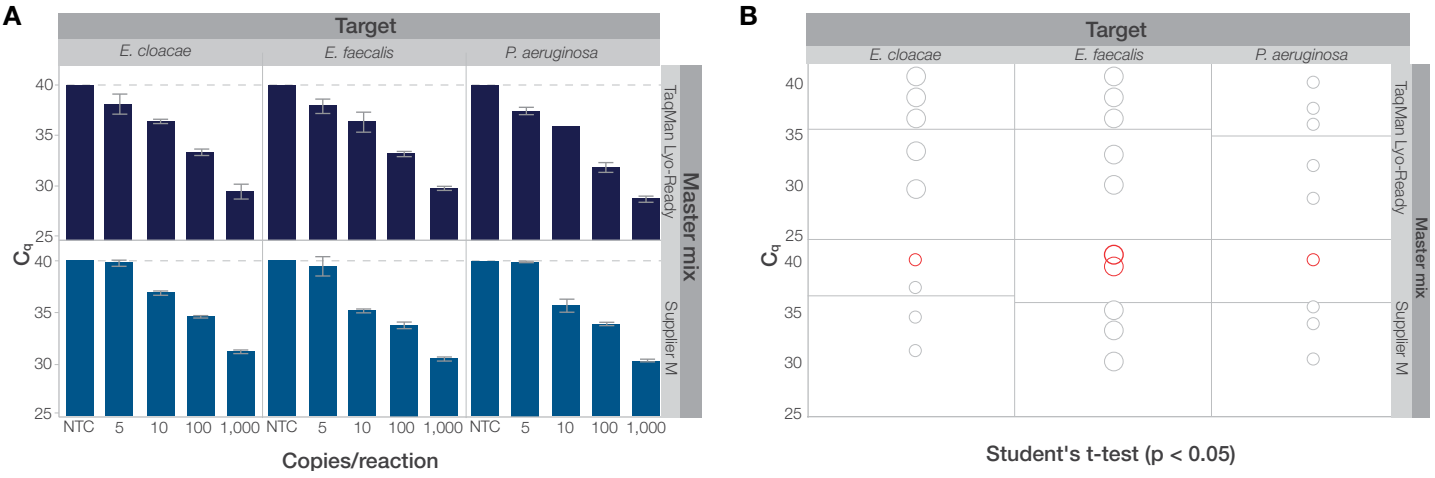


Figure 7. Limit of detection. The LOD of TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient was compared to that of another supplier's master mix. For each master mix, three replicates containing varying amounts of DNA (0, 5, 10, 100, or 1,000 copies/reaction) were amplified using a custom 5-plex assay (targeting 4 common mastitis-causing bacteria plus Xeno DNA). **(A)** TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient was the only master mix that successfully differentiated between 0 and 5 copies of DNA for the 3 targets shown. **(B)** A student's t-test confirms that, for all targets shown, there is a significant difference between serial dilution data from the TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient (black circles), but not for the other commercial master mix (overlapping red circles).

Specifications	
Concentration	2.5X
Sample type	DNA
Reaction speed	Standard and fast modes
Detection method	Primers and probe
Multiplexing	Up to 5 targets
Benchtop stability	4 hours
Dynamic range*	7 orders of magnitude (10 to 10^8 copies per reaction)

Limit of detection	Differentiates between 0 and 5 copies of DNA
Passive reference dye	None
Excipient	Premixed
Applications	Lyophilization, qPCR
Shipping conditions	Dry ice

* Dynamic range is a function of the assay and template concentration in the sample, as well as the formulation of the master mix; thus, individual results may vary.

Ordering information

Description	Size	Sufficient for	Cat. No.
TaqMan 2.5X Lyo-Ready qPCR Master Mix With Excipient	5 mL	500 reactions (25 µL)	C14081B001
	50 mL	5,000 reactions (25 µL)	C14081B002

For a sample, quote, or a customized solution, please reach out to custom.solutions@thermofisher.com.

Learn more at **thermofisher.com/qpcr-lyoready**