



qPCR

# TaqMan 2.5X Lyo-Ready 1-Step qPCR Master Mix With Excipient

applied biosystems

The Applied Biosystems™ TaqMan™ 2.5X Lyo-Ready 1-Step 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 ensure 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.

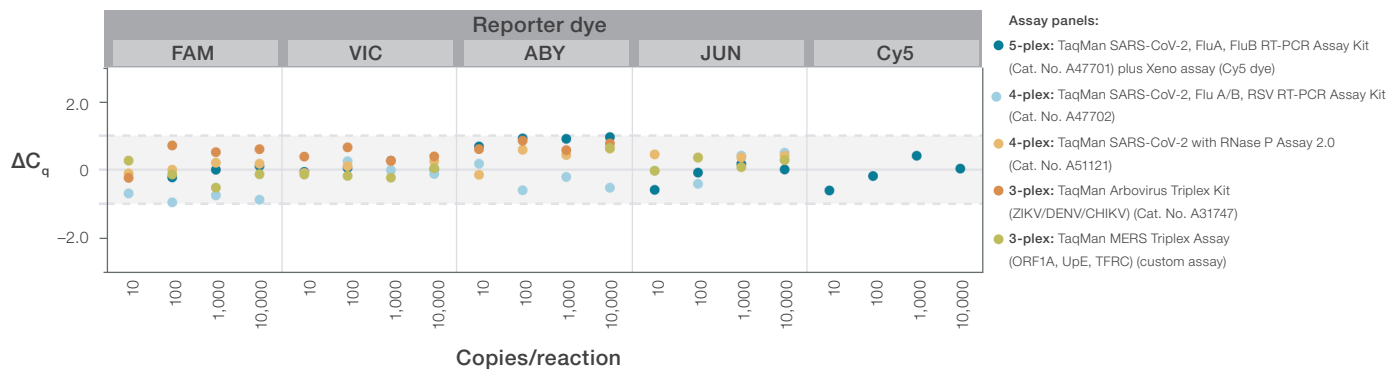


### Benefits of TaqMan 2.5X Lyo-Ready 1-Step qPCR Master Mix With Excipient

- **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.



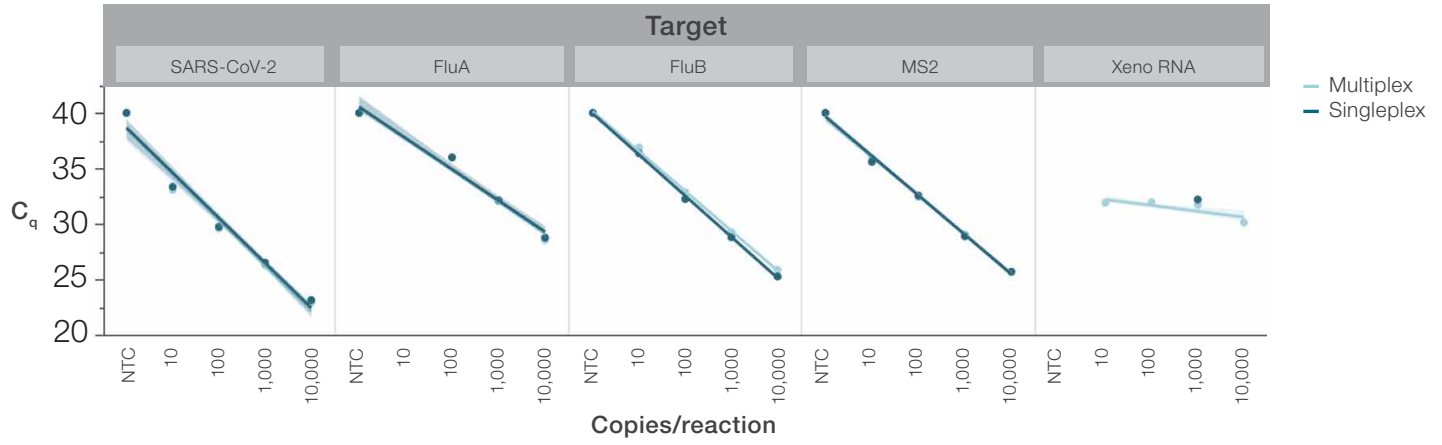
**Figure 1. Optimized for lyophilization (performance before and after lyophilization).** The performance of the TaqMan 2.5X Lyo-Ready 1-Step Master Mix With Excipient, before and after lyophilization, was evaluated with synthetic templates and an extended panel of TaqMan multiplex assays. For each of the assays, three replicates of a 10-fold dilution series of an RNA control were quantified using pre-lyophilized (liquid) and lyophilized master mix. To show performance changes, the  $\Delta C_q$  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 1-Step Master Mix With Excipient has  $\Delta C_q$  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 1-Step qPCR Master Mix With Excipient has been validated for multiplexing up to 5 targets in one 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.

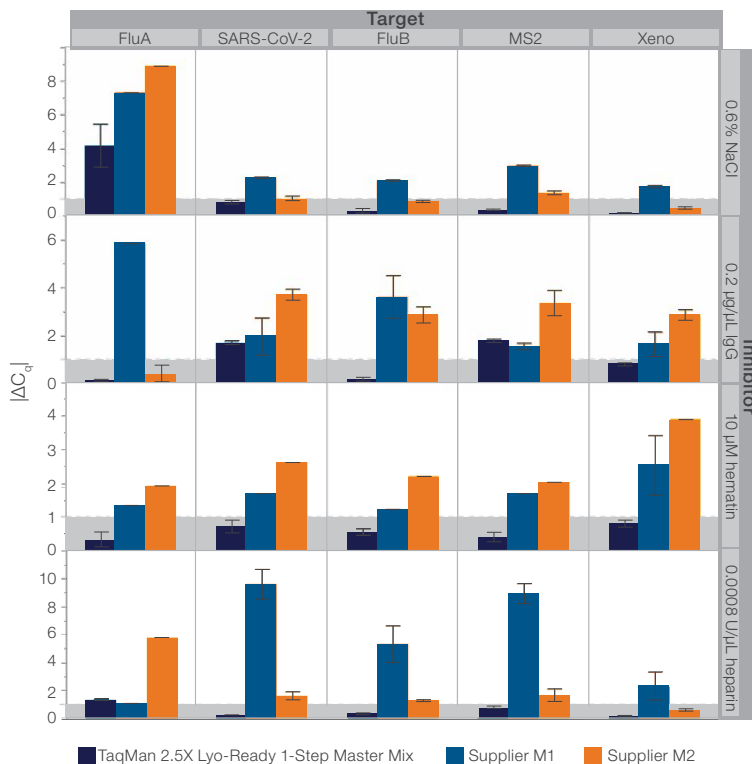
## Tolerant of inhibitors

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).

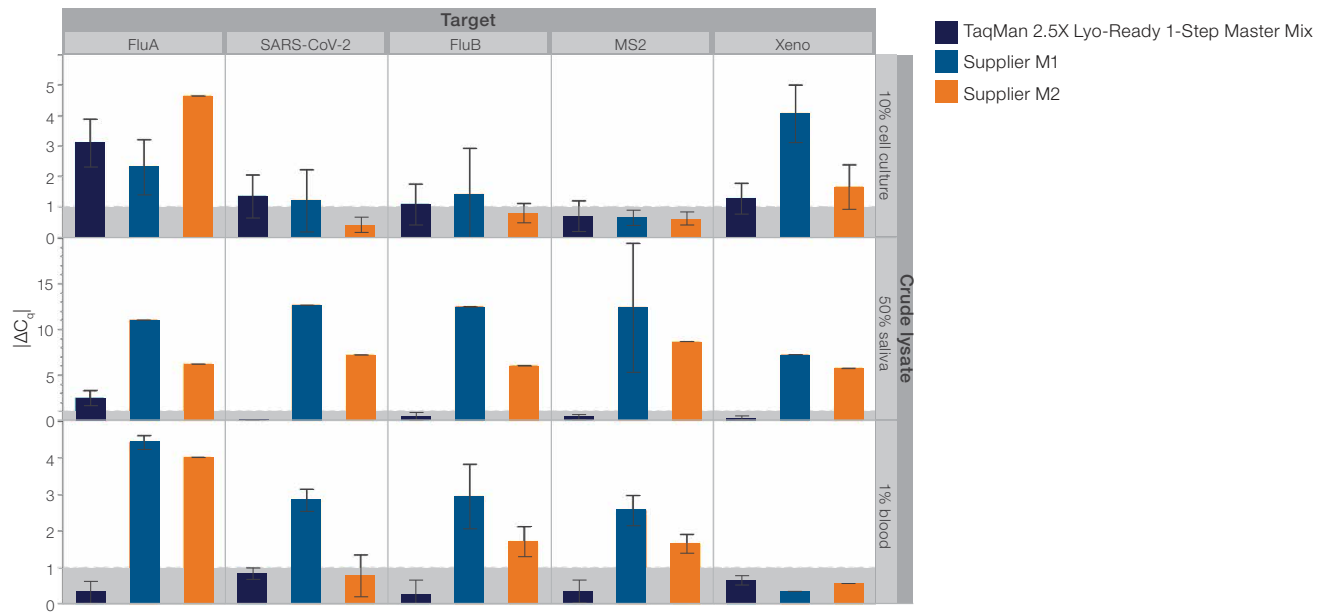


| Plex   | SARS-CoV-2 |                | FluA       |                | FluB       |                | MS2        |                | Xeno (IPC) |
|--------|------------|----------------|------------|----------------|------------|----------------|------------|----------------|------------|
|        | Efficiency | R <sup>2</sup> | Efficiency | R <sup>2</sup> | Efficiency | R <sup>2</sup> | Efficiency | R <sup>2</sup> | Slope      |
| 1-plex | 97.8%      | 0.998          | 88.9%      | 0.991          | 87.6%      | 0.996          | 98.8%      | 0.995          | 0.00       |
| 5-plex | 98.0%      | 1.000          | 85.4%      | 0.990          | 87.9%      | 0.997          | 96.5%      | 0.993          | 0.53       |

**Figure 2. Optimized for multiplexing.** The performance of TaqMan 2.5X Lyo-Ready 1-Step Master Mix With Excipient in multiplex assays was assessed using singleplex and 5-plex reactions. For each target, a 10-fold RNA dilution series was prepared, with four replicates of each dilution. A constant concentration of Xeno RNA, as an internal positive control (IPC), was added across all dilutions. The figure illustrates that TaqMan 2.5X Lyo-Ready 1-Step Master Mix With Excipient maintains comparable performance between singleplex and 5-plex reactions for all of the microbial targets, while maintaining the same C<sub>q</sub> for the IPC, as indicated by the linear amplification plots, qPCR efficiencies, and R<sup>2</sup> values.



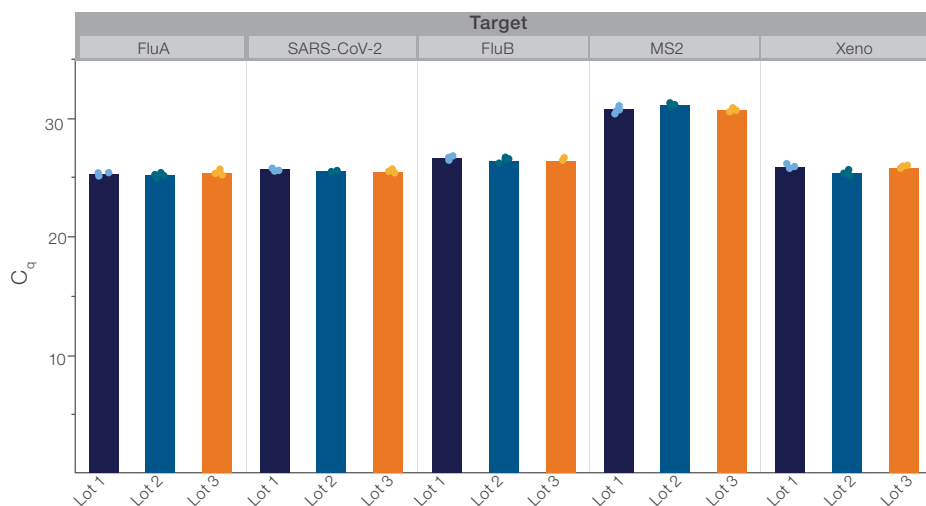
**Figure 3. High inhibitor tolerance.** The inhibitor tolerance of TaqMan 2.5X Lyo-Ready 1-Step Master Mix With Excipient was compared to master mixes from other suppliers. For each master mix, four replicates of samples containing 10,000 copies/reaction of artificial template were amplified using a 5-plex assay (Applied Biosystems™ TaqMan™ SARS-CoV-2, FluA, FluB RT-PCR Assay Kit (Cat. No. A47701) plus Xeno assay with Cy<sup>5</sup> reporter dye), both with and without the following inhibitors: NaCl (0.6%), IgG (0.2 μg/μL), hematin (10 μM), or heparin (0.0008 U/μL). To show performance changes in the presence of inhibitors, the absolute value of  $\Delta C_q$  ( $|\Delta C_q|$ ) 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  $\leq 1$  considered not significant (dashed gray line). As demonstrated in the plot, TaqMan 2.5X Lyo-Ready 1-Step Master Mix With Excipient has low  $|\Delta C_q|$  values across targets, indicating comparable performance between inhibited and non-inhibited samples. The other two master mixes have substantially higher  $|\Delta C_q|$  values for all targets, indicating they have lower inhibitor tolerances and are 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 1-Step Master Mix With Excipient to crude lysates of cell culture (K562 cell line), saliva, and whole blood was compared to those of other suppliers' master mixes. For each master mix, three replicates of samples containing 10,000 copies/reaction of artificial template were amplified using a 5-plex assay (TaqMan SARS-CoV-2, FluA, FluB RT-PCR Assay Kit (Cat. No. A47701) plus Xeno assay with Cy5 reporter dye), both with and without crude lysate added: 10% of the reaction volume of cell culture crude lysate, 50% for saliva, and 1% for blood. The crude lysates were obtained using the Invitrogen™ Cells-to-C<sub>T</sub>™ 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 value of  $\Delta C_q$  ( $|\Delta C_q|$ ) 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  $\leq 1$  considered not significant (dashed gray line). As demonstrated in the plot, TaqMan 2.5X Lyo-Ready 1-Step Master Mix With Excipient has low  $|\Delta C_q|$  values across targets in most cases, indicating comparable performance between inhibited and non-inhibited samples. The other two master mixes have substantially higher  $|\Delta C_q|$  values for all targets, indicating they have lower inhibitor tolerances and are more susceptible to the effects of inhibitors on performance.

### Lot-to-lot consistency

TaqMan 2.5X Lyo-Ready 1-Step Master Mix With Excipient has been optimized for highly reproducible detection from a wide variety of samples (Figure 4). Figure 5 shows consistent  $C_q$  results obtained from three unique lots across multiple assays, to maximize confidence in your results and highlight the product's exceptional lot-to-lot consistency.



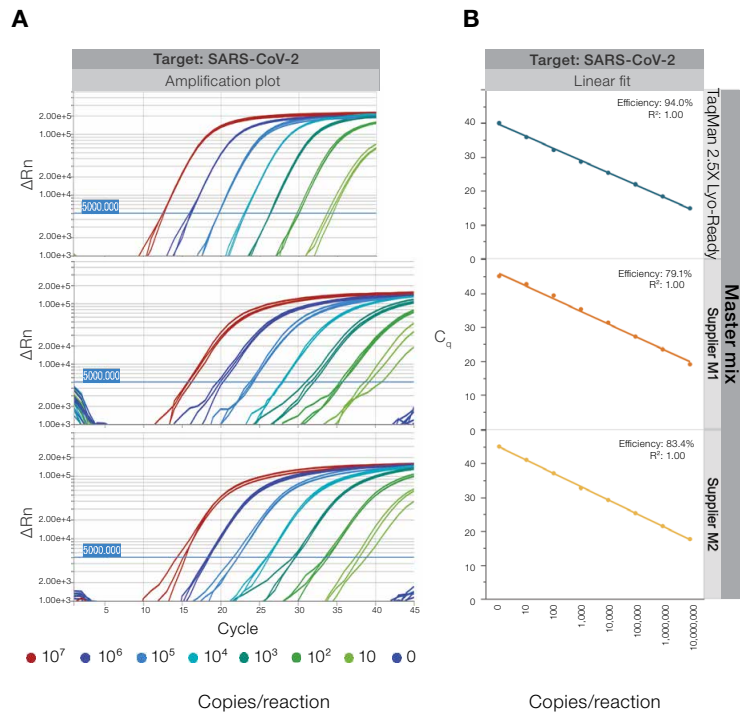
**Figure 5. Exceptional lot-to-lot performance consistency.** The consistent performance of four replicates of three different lots of TaqMan 2.5X Lyo-Ready 1-Step Master Mix With Excipient was demonstrated using 10,000 copies/reaction of RNA template and a 5-plex assay (TaqMan SARS-CoV-2, FluA, FluB RT-PCR Assay Kit (Cat. No. A47701) plus Xeno assay with Cy5 reporter dye). Excellent  $C_q$  concordance is seen across the three lots for all the targets.

## Dynamic range and limit of detection (LOD)

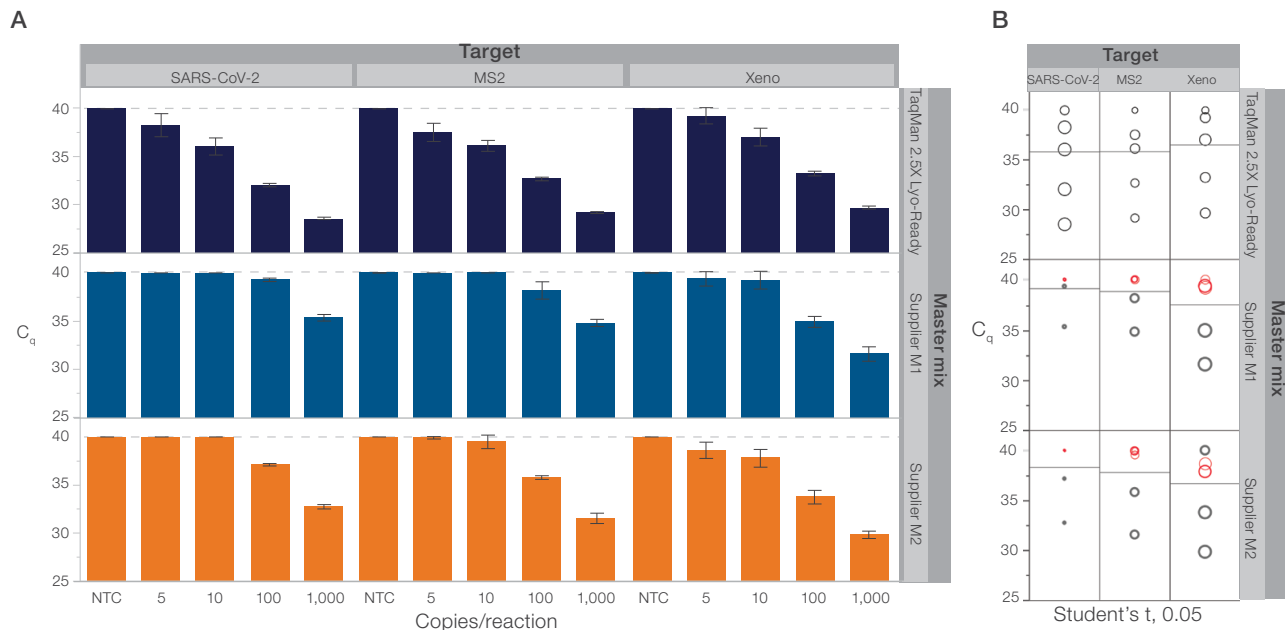
TaqMan 2.5X Lyo-Ready 1-Step Master Mix With Excipient demonstrates a wide dynamic range (Figure 6) and reliable differentiation between 0 and 5 copies of RNA (Figure 7).

## 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 6 orders of magnitude.** The linear dynamic range of TaqMan 2.5X Lyo-Ready 1-Step Master Mix With Excipient was compared to those of master mixes from other suppliers. Three replicates of a 10-fold RNA dilution series and a 5-plex assay (TaqMan SARS-CoV-2, FluA, FluB RT-PCR Assay Kit (Cat. No. A47701) plus Xeno assay with Cy5 reporter dye) were tested. **(A)** Amplification curves and **(B)** linearized amplification data with PCR efficiency and  $R^2$  are shown for the SARS-CoV-2 target of the 5-plex assay. The results demonstrate consistent amplification across seven 10-fold dilution points for TaqMan 2.5X Lyo-Ready 1-Step Master Mix With Excipient. Lower PCR efficiencies are observed for the other suppliers, below the acceptable range of 90%–110%.



**Figure 7. Limit of detection.** The LOD of TaqMan 2.5X Lyo-Ready 1-Step Master Mix With Excipient was compared to those of master mixes from other suppliers. For each master mix, four replicates containing varying amounts of RNA (0, 5, 10, 100, or 1,000 copies/reaction) were amplified using a 5-plex assay (TaqMan SARS-CoV-2, FluA, FluB RT-PCR Assay Kit (Cat. No. A47701) plus Xeno assay with Cy5 reporter dye). **(A)** TaqMan 2.5X Lyo-Ready 1-Step qPCR Master Mix With Excipient was the only master mix that successfully differentiated between 0 and 5 copies of RNA for the 3 targets shown. **(B)** The 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 1-Step Master Mix With Excipient (black circles), but not for the other commercial master mixes (overlapping red circles).

## Specifications

|                       |   |
|-----------------------|---|
| Concentration         | 2.5X  |
| Sample types          | RNA, DNA  |
| Reaction speed        | Standard and fast modes   |
| Detection method      | Primers and probe   |
| Multiplexing          | Up to 5 targets   |
| Benchtop stability    | 4 hours   |
| Dynamic range*        | 6 orders of magnitude (10 to 10 <sup>7</sup> copies per reaction) |
| Limit of detection    | Differentiates between 0 and 5 copies of RNA                      |
| 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   | Quantity | Sufficient for          | Cat. No.   |
|---|----------|-------------------------|------------|
| TaqMan 2.5X Lyo-Ready 1-Step qPCR Master Mix With Excipient | 5 mL     | 500 reactions (25 µL)   | C14031B001 |
|   | 50 mL    | 5,000 reactions (25 µL) | C14031B002 |

For a sample, quote, or a customized solution, please reach out to [custom.solutions@thermofisher.com](mailto:custom.solutions@thermofisher.com)

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