

TaqPath 1-Step Multiplex Master Mix

Developed for demanding RNA virus detection and high-throughput gene expression analysis protocols, Applied Biosystems™ TaqPath™ 1-Step Multiplex Master Mix is designed to deliver sensitive and reproducible detection of up to four RNA (or DNA) targets in a single multiplex RT-qPCR reaction. While the single-tube, 4X format facilitates easy reaction setup (Figure 1), the reproducible performance even in the presence of inhibitors provides confidence in results.

We offer two formulations of this master mix, which is compatible with Applied Biosystems™ multiplex assays:

- TaqPath 1-Step Multiplex Master Mix, which includes MUSTANG PURPLE™ passive reference dye
- TaqPath 1-Step Multiplex Master Mix (No ROX), which has no passive reference dye

Both mixes enable measurement of JUN™ dye or other dyes with a similar emission wavelength, in the channel typically used to measure ROX™ dye. These two

formulations join our line of Applied Biosystems™ TaqPath™ General Purpose Reagents—qPCR and RT-qPCR master mixes manufactured in an ISO 13485–certified facility with stringent production and process controls to help ensure lot-to-lot consistency. With 15 years of technology leadership in real-time PCR, we are committed to continually providing laboratories with trusted, versatile, and innovative tools for the future of molecular diagnostics.

Features of TaqPath 1-Step Multiplex Master Mix:

- Ability to detect up to 4 targets in one reaction
- High sensitivity to detect low-copy targets with reproducible C_t results
- Wide dynamic range compatible with multiplexing applications*
- Tolerance of inhibitors commonly found in clinical samples
- Manufactured with stringent process controls to help ensure lot-to-lot consistency

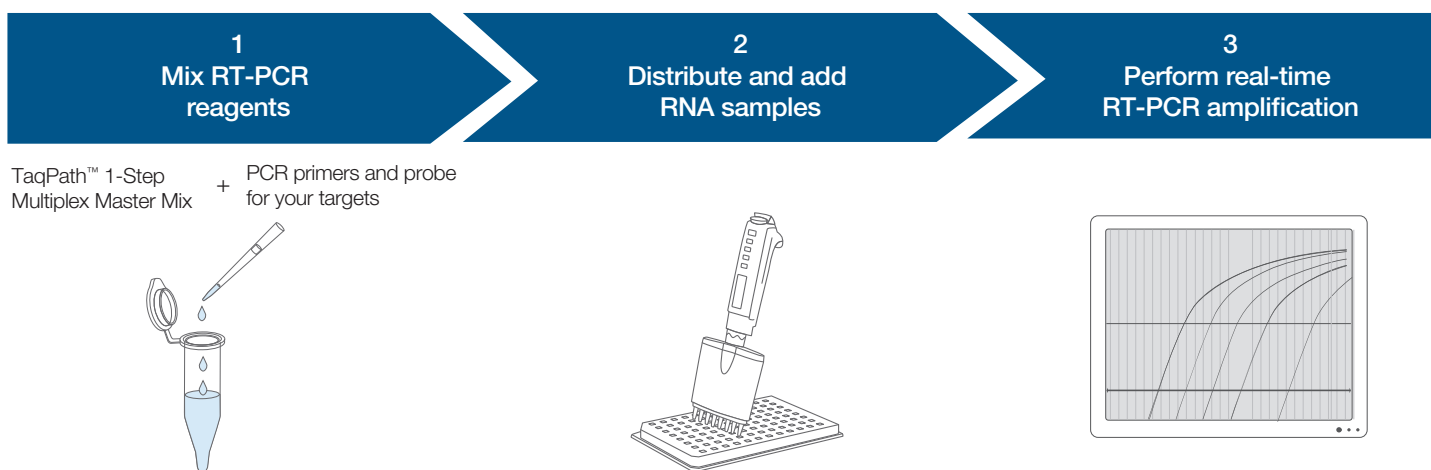


Figure 1. RT-qPCR workflow using TaqPath 1-Step Multiplex Master Mix.

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

High sensitivity

We understand the importance of reproducible detection for low-titer pathogens or transcripts in clinical diagnostic testing, which is why TaqPath 1-Step Multiplex Master Mix has been optimized as a higher-concentration (4X) master mix that allows input of more sample into each reaction, increasing sensitivity even in low-volume reactions. Figure 2 shows consistent C_t results obtained using both master mixes (the mix without the ROX dye and the mix containing MUSTANG PURPLE dye) to detect inputs of 10 copies of an Ebola virus RNA target.

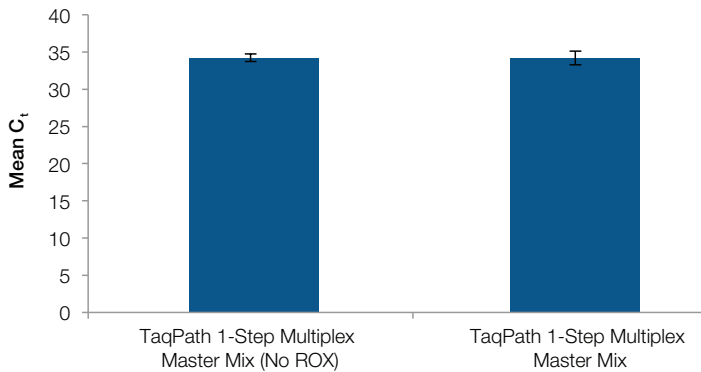


Figure 2. Reliable low-copy detection. Samples containing 10 copies of Ebola virus RNA target were amplified using each of the TaqPath 1-Step Multiplex Master Mixes and an assay labeled with FAM™ dye. Error bars show the standard deviation from 12 replicates.

Wide linear dynamic range with both RNA and DNA samples

TaqPath 1-Step Multiplex Master Mix has been optimized to provide high specificity and dynamic range for both RNA and DNA targets. Since virology labs often test for both RNA and DNA viruses, TaqPath 1-Step Multiplex Master Mix is designed to use a single protocol to assay both types of nucleic acid. This input flexibility can help streamline the number of different workflows, especially in virology labs, to help improve efficiency. Figure 3 demonstrates the excellent PCR linearity across an input range of 6 orders of magnitude for both the MUSTANG PURPLE passive reference mix and the No ROX mix. Figure 4 demonstrates a linear dynamic range of 6 orders of magnitude with both RNA and DNA targets.

Optimized for multiplexing

TaqPath 1-Step Multiplex Master Mix is compatible with multiplexing of reactions, allowing additional exogenous or endogenous controls or targets to be run simultaneously for quality control or increased efficiency. Both versions of the mix can be used in conjunction with Applied Biosystems™ TaqMan™ probes with FAM, VIC™, ABY™, and JUN reporter dye labels and QSY™ quenchers to provide detection of 4 targets in a single reaction. These reporter

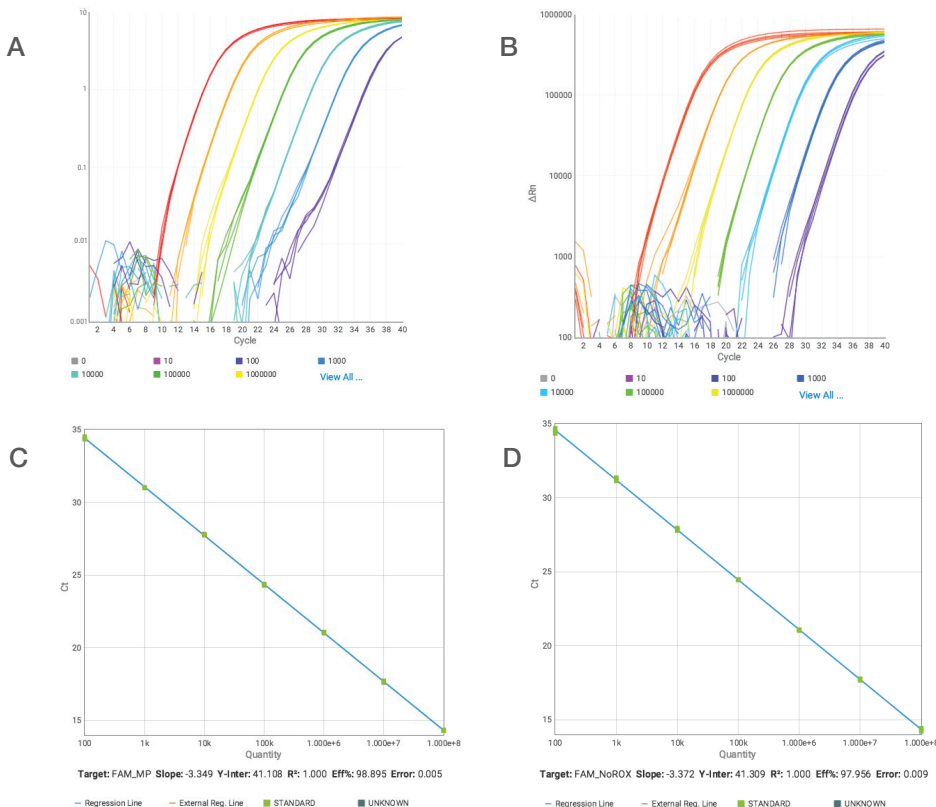


Figure 3. Excellent dynamic range of TaqPath 1-Step Multiplex Master Mix. (A) Amplification plot for a 6-log dilution series of Ebola virus RNA with the master mix containing MUSTANG PURPLE dye and a TaqMan™ Assay, on the Applied Biosystems™ QuantStudio™ 7 Real-Time PCR System. $R^2 = 1.0$, 98.9% efficiency. (B) Amplification plot for the same 6-log dilution series and TaqMan™ Assay with the No ROX master mix $R^2 = 1.0$, 97.6% efficiency. (C, D) Standard curves derived from the plots in A and B, respectively.

dyes are optimized to work together—in both formulations of the master mix—with minimal spectral overlap for optimal performance. Figure 4 demonstrates the performance of TaqPath 1-Step Multiplex Master Mix in 4-plex reactions with DNA and RNA targets.

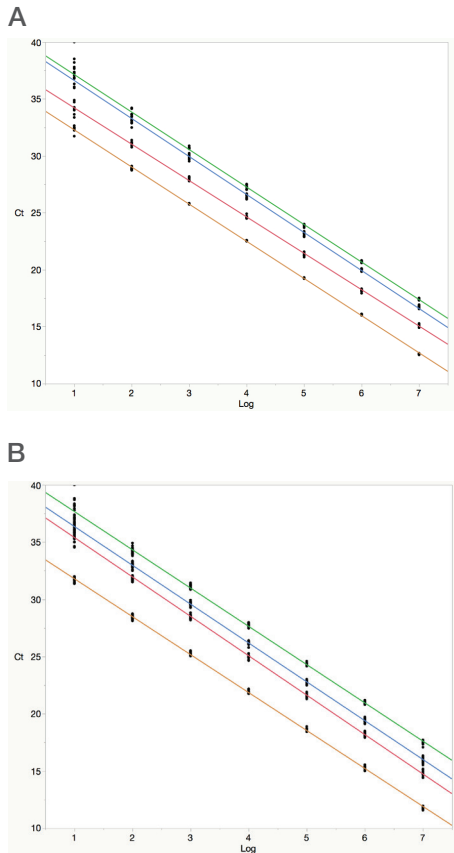


Figure 4. TaqPath 1-Step Multiplex Master Mix is optimized for multiplexing with both RNA and DNA targets. (A) Amplification results for a 4-plex reaction using human cDNA over 6 orders of magnitude, TaqPath 1-Step Multiplex Master Mix, and assays for CD44 (red), CYC1 (green), TMSB10 (orange), and G6PD (blue). $R^2 = 1.0$ for all targets. **(B)** Amplification results for a 4-plex reaction using human RNA over 6 orders of magnitude, TaqPath 1-Step Multiplex Master Mix, and assays for CD44 (red), CYC1 (green), TMSB10 (orange), and G6PD (blue). $R^2 = 1.0$ for all targets. The assay probes were labeled with FAM, VIC, ABY, and JUN dyes, respectively.

Inhibitor tolerance

Unlike other master mixes on the market, the unique proprietary formulation of TaqPath 1-Step Multiplex Master Mix allows robust performance even in the presence of substances that normally inhibit PCR, such as hematin and heparin, increasing your confidence when working with a variety of complex clinical samples. Figure 5 depicts the enhanced performance of TaqPath 1-Step Multiplex Master Mix in the presence of two common inhibitors, in comparison with three competitors' 1-step kits.

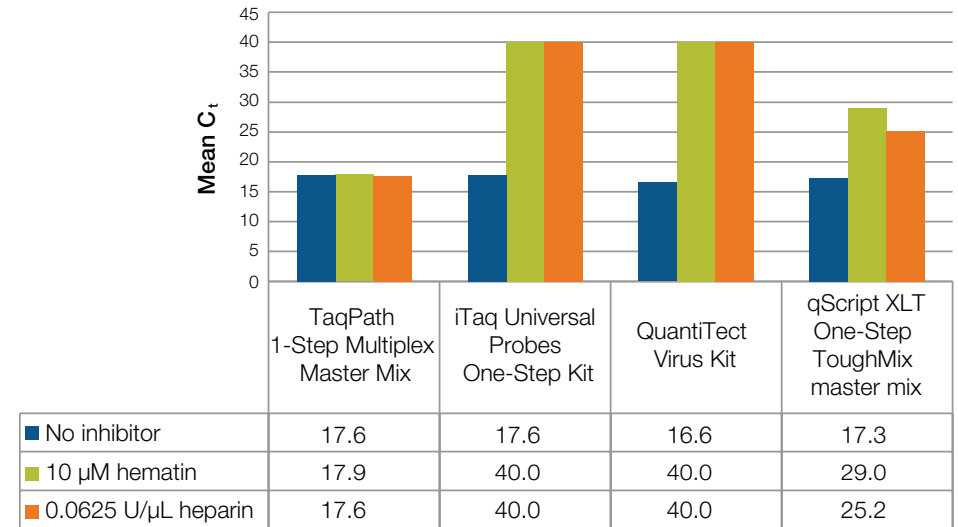


Figure 5. Inhibitor tolerance of TaqPath 1-Step Multiplex Master Mix and competitor kits. Two inhibitors (hematin and heparin) were added to RT-qPCR reactions run on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System to assess the magnitude of C_t shift caused by these inhibitors. C_t values for reactions without and with inhibitors are shown.

Manufacturing production and process controls

With established controls from purchasing through QC release, the manufacturing of TaqPath™ products is designed to deliver consistent performance lot after lot. Figure 6 demonstrates that lot-to-lot consistency of C_t is preserved across multiple assays with different attributes and input target levels.

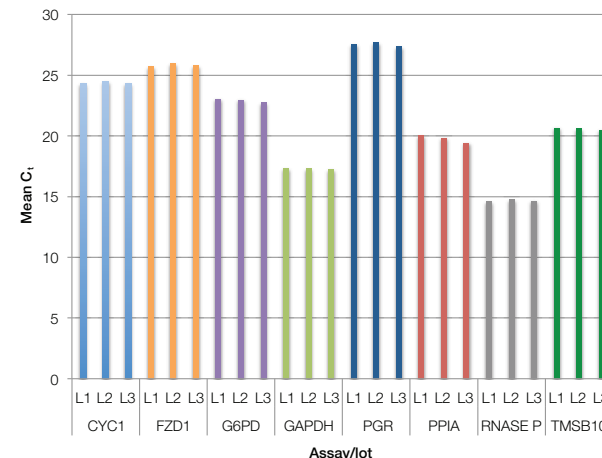


Figure 6. C_t consistency across multiple assays for three unique lots of TaqPath 1-Step Multiplex Master Mix. Total RNA was amplified using a panel of gene expression assays and three distinct lots of TaqPath 1-Step Multiplex Master Mix. Excellent C_t concordance is seen across the three lots for a representative subset of the assays used.

Broad instrument compatibility

The TaqPath 1-Step Multiplex Master Mixes can be used in either Fast or standard cycling conditions with equivalent performance across a wide variety of real-time PCR systems, including the Applied Biosystems™ qPCR platforms (e.g., 7500, 7500 Fast, and 7500 Fast Dx; QuantStudio™ 5, 6, 7, 12K Flex, and Dx; ViiA™ 7).**

General purpose reagents

The TaqPath master mixes are general purpose reagents manufactured in an ISO 13485–certified facility and labeled “For Laboratory Use.” The TaqPath™ multiplex master mixes

are part of the TaqPath™ General Purpose Reagents for qPCR and 1-Step RT-qPCR master mixes, which include:

- TaqPath™ qPCR Master Mix, CG—includes ROX passive reference dye
- TaqPath™ 1-Step RT-qPCR Master Mix—includes ROX passive reference dye
- TaqPath 1-Step Multiplex Master Mix—includes MUSTANG PURPLE passive reference dye
- TaqPath 1-Step Multiplex Master Mix (No ROX)—does not include a passive reference dye

Ordering information

Product	Unit size	Number of reactions (20 µL)	Cat. No.
TaqPath 1-Step Multiplex Master Mix (No ROX)	1 x 0.5 mL	100	A28521
TaqPath 1-Step Multiplex Master Mix (No ROX)	5 x 1 mL	1,000	A28522
TaqPath 1-Step Multiplex Master Mix (No ROX)	1 x 10 mL	2,000	A28523
TaqPath 1-Step Multiplex Master Mix	1 x 0.5 mL	100	A28525
TaqPath 1-Step Multiplex Master Mix	5 x 1 mL	1,000	A28526
TaqPath 1-Step Multiplex Master Mix	1 x 10 mL	2,000	A28527

For more information, go to thermofisher.com/taqpath

** TaqMan Assays and probes, Applied Biosystems 7500, 7500 Fast, and ViiA 7 Real-Time PCR Systems, and QuantStudio 5, 6, 7, and 12K Flex Real-Time PCR Systems are for Research Use Only. TaqPath master mixes are For Laboratory Use.