Confidence in clinical testing: TaqPath general purpose reagents

Introduction

The Applied Biosystems™ TaqPath™ general purpose reagents are designed for use in molecular diagnostics development and testing. With stringent manufacturing quality and excellent performance, these real-time PCR master mixes are designed to deliver confidence and reliability for even your most demanding applications. Formulations are available to support both qPCR and one-step RT-qPCR using 5'-nuclease assays in singleplex or multiplex format. Each reagent is manufactured and labeled in accordance with requirements for general purpose reagents, and is functionally tested to help ensure lot-to-lot reproducibility for C_t consistency and a wide dynamic range. With 15 years of technology leadership in real-time PCR, we are committed to continually providing clinical laboratories with trusted, versatile, and innovative tools for the future of molecular diagnostics.

Features of the TaqPath™ master mixes include:

- High sensitivity to detect low-copy targets with reproducible C_t results
- Wide dynamic range compatible with multiplexing applications*
- Tolerant of inhibitors commonly found in clinical samples
- Manufactured in an ISO-13485 facility to help ensure excellent manufacturing consistency
- Labeled "For Laboratory Use"

TaqPath general purpose reagent	Application	Passive reference dye
TaqPath ProAmp Master Mix	Genotyping and copy number variation (CNV)	ROX
TaqPath ProAmp Multiplexing Master Mix	Genotyping and CNV (multiplexing)	Mustang Purple
TaqPath qPCR Master Mix, CG	qPCR	ROX
TaqPath 1-Step qRT-PCR Master Mix	One-step RT-qPCR	ROX
TaqPath 1-Step Multiplex Master Mix	One-step RT-qPCR	Mustang Purple
TaqPath 1-Step Multiplex Master Mix (No ROX)	One-step RT-qPCR	None

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TaqPath ProAmp Master Mix

The Applied Biosystems™ TaqPath™ ProAmp™ Master Mix is a versatile master mix developed for high-throughput genotyping and copy number assay protocols requiring accurate results, even for samples containing PCR inhibitors (Figure 1). It is designed to deliver sensitive and reproducible results from genomic DNA targets in a single or multiplex reaction.

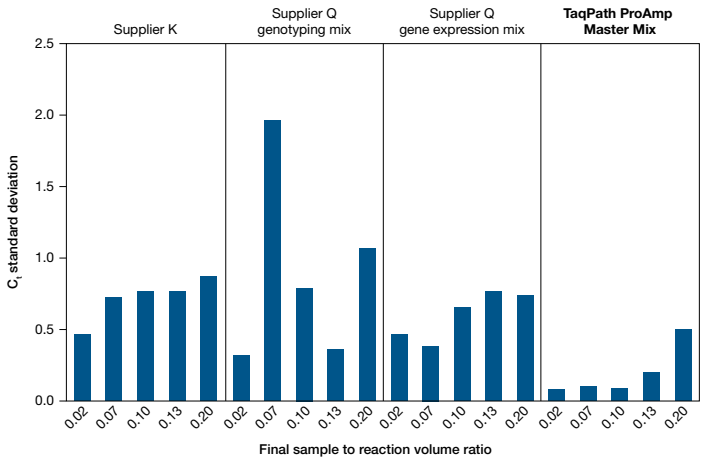


Figure 1. Mean C_t standard deviation of TaqPath ProAmp Master Mix vs. other commercial master mixes. Results are based on eight replicate qPCR reactions using an RNase P assay and increasing ratios of blood sample volume to reaction volume as shown on the x-axis. TaqPath ProAmp Master Mix has a low standard deviation in C_t values relative to the other commercial master mixes at the recommended 0.02 input ratio and across all ratios up to 20% of reaction volume.

We offer two formulations of this master mix, which are compatible with Applied Biosystems™ TaqMan® multiplex assays**:

- TaqPath ProAmp Master Mix, which includes ROX™ passive reference dye
- Applied Biosystems™ TaqPath™ ProAmp™ Multiplex Master Mix, which includes Mustang Purple™ passive reference dye

Both mixes enable measurement of FAM™, VIC™, and ABY™ dyes or other dyes with similar emission wavelengths. The multiplex version also allows the use of JUN™ dye, or other dyes with similar emission wavelengths, in the channel typically used to measure ROX dye for duplex single nucleotide polymorphism (SNP) genotyping and other higher multiplex applications.

Features of TaqPath ProAmp Master Mix:

- **Exceptional data quality**—high specificity, dynamic range*, and reproducibility for genotyping and copy number determination
- **Tolerance to inhibitors**—compatible with samples prepared from human or animal sources (buccal swabs, blood, and card punches)
- **72 hr pre-PCR benchtop stability**—proprietary Applied Biosystems™ Dual-Lock™ *Taq* DNA Polymerase with Dual-Lock™ hot-start mechanism for setup on automated workflow
- **Multiplexing breadth**—detect up to 4 targets per reaction
- **Excellent manufacturing quality**—manufactured in an ISO 13485–certified facility

TaqPath qPCR Master Mix, CG

Applied Biosystems™ TaqPath™ qPCR Master Mix, CG is designed, with functionally of all lots tested, to help ensure lot-to-lot reproducibility for consistent C_t values and dynamic range across a wide variety of assays. With stringent quality and premier performance, TaqPath qPCR Master Mix, CG is a superior choice for your diagnostics testing or development needs.

Features of TaqPath qPCR Master Mix, CG include:

- Efficient and linear detection up to 7 orders of magnitude with gene expression or miRNA assays*
- Enables reliable detection of low-copy templates with reproducible C_t values
- Robust multiplexing performance with exogenous or endogenous targets
- Manufactured with stringent production and process controls in an ISO 13485–certified facility to help ensure lot-to-lot consistency
- Labeled “For Laboratory Use”

TaqPath qPCR Master Mix, CG is a 2X formulation designed for gene expression and miRNA analysis. It contains thermostable, fast DNA polymerase, uracil N-glycosylase (UNG), dNTPs with dUTP, ROX dye (passive reference), and optimized buffer components for maximum robustness and reproducibility.

Validated with a breadth of workflows

TaqPath qPCR Master Mix, CG has been validated to provide high specificity and dynamic range for use in multiple real-time PCR applications. The formulation can be used in either Fast or standard cycling conditions on a wide variety of qPCR platforms. Figure 2 demonstrates the excellent PCR linearity over a template input range of 7 orders of magnitude when used in both gene expression and miRNA assays.

In addition, TaqPath qPCR Master Mix, CG has been engineered to retain consistent performance in preassembled reactions for up to 48 hours. The stability of this mix allows users of high-throughput liquid handling systems to achieve results on the last plate that parallel those on the first plate (Figure 3). TaqPath qPCR Master Mix, CG was tested in an internal benchmarking study against similar master mixes from other suppliers and demonstrated equivalent or better sensitivity and dynamic range across a variety of targets (Table 1).

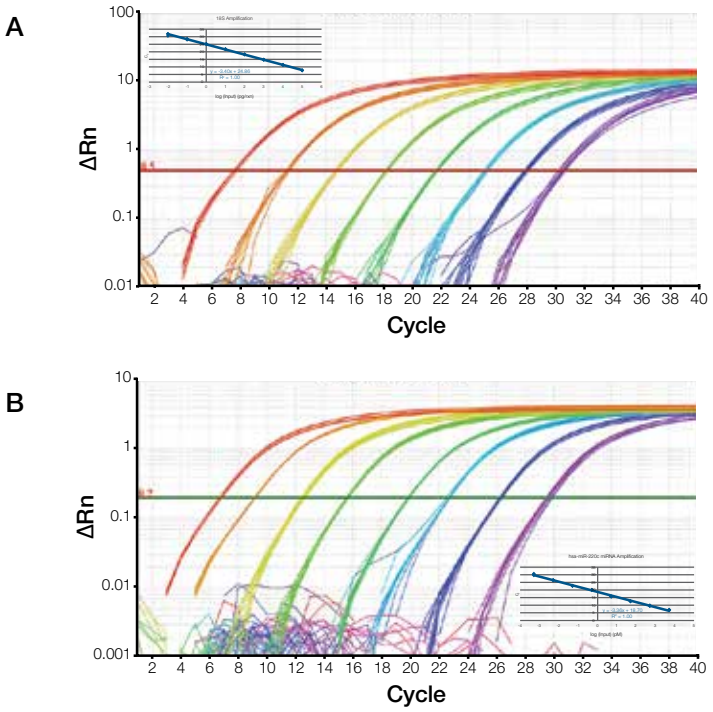
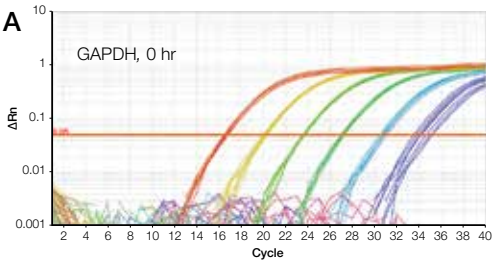
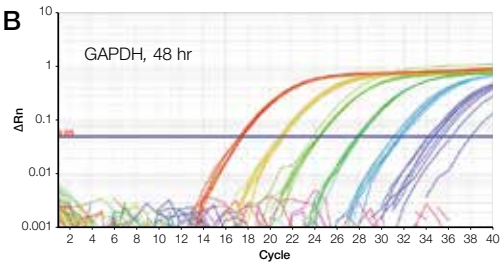


Figure 2. Excellent dynamic range of TaqPath qPCR Master Mix, CG. Representative amplification plots using the Applied Biosystems™ ViiA™ 7 Real-Time PCR System** for **(A)** an 18S assay with human cDNA dilution series or **(B)** an hsa-miR-220c miRNA assay with an artificial template dilution series.





C

Time	0 hr	48 hr
PCR efficiency	97.4	95.1

Final concentration (pg/reaction)	0 hr	48 hr
100,000	17.1	17.5
10,000	20.7	21.2
1,000	23.6	24.2
100	27.3	27.8
10	30.7	31.2
1	34.9	34.6

Figure 3. Benchtop stability of TaqPath qPCR Master Mix, CG. The GAPDH gene expression assay was run upon assembly: **(A)** at time 0 and **(B)** after 48 hours of incubation at 24°C. **(C)** The results after 48 hours show excellent PCR efficiency, R^2 values, and C_t values, compared to that obtained at time 0.

Table 1. Dynamic range comparison between TaqPath qPCR Master Mix, CG and master mixes from other leading suppliers. Comparison of detection range (expressed as number of 10-fold dilutions that yield a standard curve) for a panel of various assays is shown. The criteria for detection were a PCR efficiency between 85% and 115%, and R^2 values >0.98. Each master mix was tested using cDNA and run according to suppliers' respective recommended protocols. Reactions (2–4 replicates) were run on the ViiA 7 Real-Time PCR System.**

Assay	TaqPath qPCR mix Fast mode ~44 min	Supplier R Fast mode ~66 min	Supplier Q standard mode ~95 min
ACTB	7	7	7
ANP32B	5	3	5
APOA1	7	6	6
FOXD1	4	5	4
HIST1H3F	6	5	5
TMX1	5	3	5
UBC	7	6	7

Reproducible, sensitive detection

We understand the importance of reliability in detecting low-copy targets for your test quality and data interpretation. TaqPath qPCR Master Mix, CG helps generate significant and reproducible C_t values for detection of ≤10 copies. Figure 4 shows the consistent C_t values obtained from three distinct lots when detecting 10-copy inputs. This lot-to-lot consistency of C_t values is preserved across multiple assays with different attributes and detection of different levels of expression to maximize confidence in your results (Figure 5).

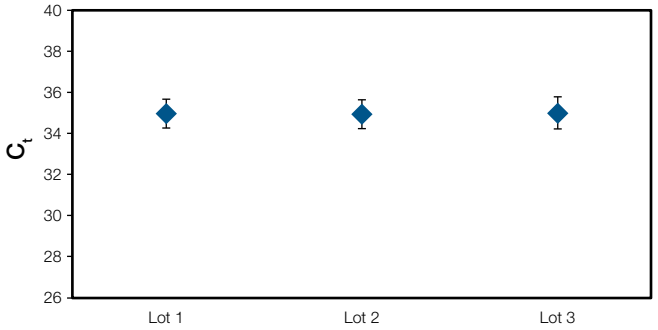


Figure 4. Reliable low-copy detection. A sample containing 10 copies of human DNA was amplified using three distinct lots of TaqPath qPCR Master Mix, CG and an RNase P assay.

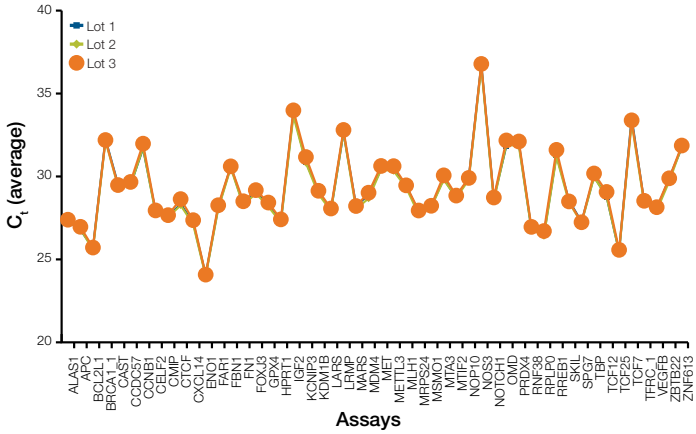


Figure 5. Consistency of C_t values across multiple assays for three lots of TaqPath qPCR Master Mix, CG. Human cDNA was amplified using a panel of 96 gene expression assays and three distinct lots of TaqPath qPCR Master Mix, CG. Excellent C_t concordance is seen across the three lots for a representative subset of the assays used.

Optimized for multiplexing

Simultaneous amplification of multiple assays can be beneficial not only as a control to normalize and detect anomalies in experiments, but also to improve efficiency and cost savings for labs. TaqPath qPCR Master Mix, CG has been optimized so that the concentrations of enzymes and other components facilitate multiplexing while preserving specificity, and it is validated for performance of duplex reaction in each manufacturing lot (Figure 6).

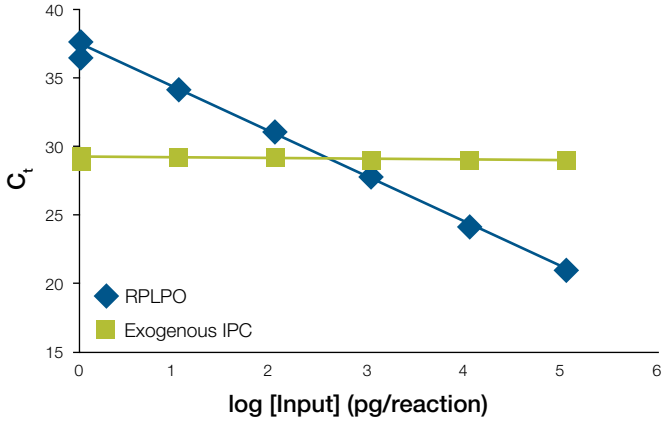


Figure 6. TaqPath qPCR Master Mix, CG is optimized for multiplexing. Amplification results are shown for a duplex reaction using human cDNA and an RPLPO (large ribosomal protein) assay with an exogenous internal positive control (IPC).

Inhibitor tolerance

Unlike other master mixes on the market, the unique proprietary formulation of TaqPath qPCR Master Mix, CG allows robust performance even in the presence of substances that normally inhibit PCR, such as heparin and hematin, thereby increasing your confidence when working with a variety of complex clinical samples. TaqPath master mix demonstrated higher tolerance to inhibitors than mixes from other suppliers in an internal benchmark study (Figure 7).

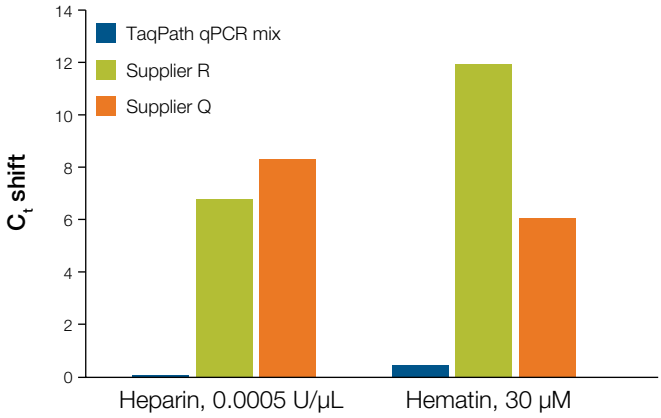


Figure 7. Comparison of inhibitor tolerance of TaqPath qPCR Master Mix, CG and kits from other suppliers. Two inhibitors of qPCR, heparin and hematin, were added to RT-qPCR reactions to assess the magnitude of shift in C_t values caused by these inhibitors. Graphs show the change in C_t from the baseline value with no inhibitor present.

TaqPath 1-Step RT-qPCR Master Mix

The Applied Biosystems™ TaqPath™ 1-Step RT-qPCR Master Mix is designed for robust, reproducible, and one-step pathogen detection and gene expression workflows. The single-tube, 4X formulation contains thermostable Moloney murine leukemia virus (M-MLV) reverse transcriptase, dNTPs, UNG, thermostable, fast DNA polymerase, and a choice of either ROX dye, Mustang Purple dye, or no passive reference dye, facilitating easy reaction setup—just add user-supplied assay and sample (Figure 8).

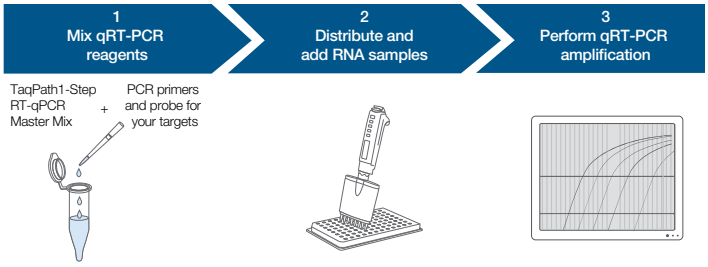


Figure 8. The simple TaqPath 1-Step RT-qPCR Master Mix workflow.

High sensitivity

To understand the importance of reproducible detection of low-titer pathogens or transcripts in clinical diagnostics testing, TaqPath 1-Step RT-qPCR 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 9 shows consistent C_t values obtained from three distinct lots when detecting 10-copy inputs of RNA target. Figure 10 demonstrates that lot-to-lot consistency of C_t values is preserved across multiple assays—with different attributes and detection of different levels of expression.

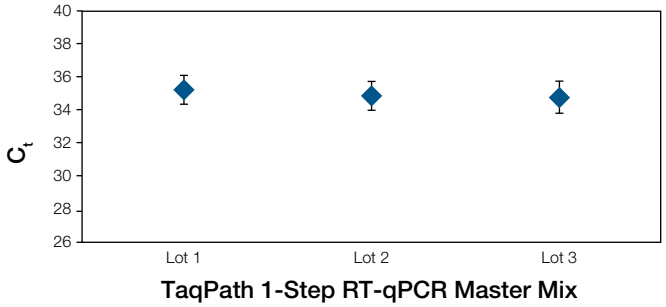


Figure 9. Reliable low-copy detection. Samples containing 10 copies of RNA target were amplified using three distinct lots of TaqPath 1-Step RT-qPCR Master Mix and an RNase P assay.

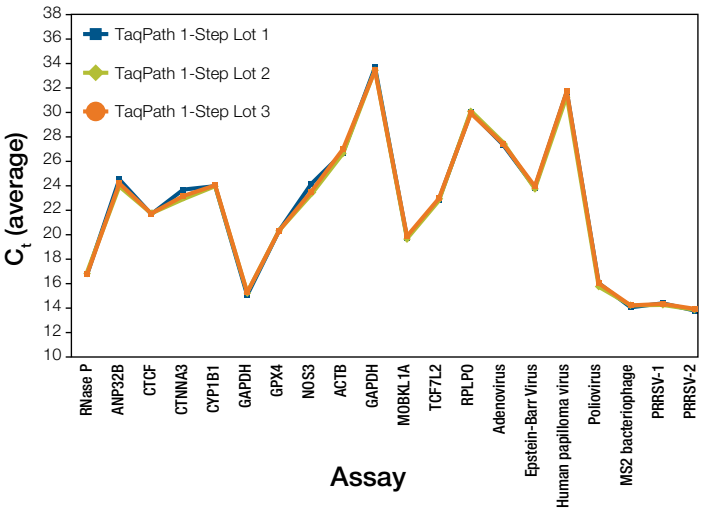


Figure 10. Consistency of C_t values across multiple assays for three unique lots of TaqPath 1-Step RT-qPCR Master Mix. Total RNA was amplified using a panel of human and viral gene expression assays and three distinct lots of TaqPath 1-Step RT-qPCR Master Mix. Excellent C_t concordance is seen across the three lots for a representative subset of the assays used.

Wide dynamic range compatible with RNA and DNA samples

TaqPath 1-Step RT-qPCR 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 RT-qPCR 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 11 demonstrates excellent PCR linearity across an input range of 6 orders of magnitude for both RNA and DNA targets.

The Applied Biosystems™ 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 dyes are optimized to work together—with Mustang Purple passive reference dye–containing mixes and No ROX mixes—with minimal spectral overlap for optimal performance. Figure 12 demonstrates TaqPath 1-Step Multiplex Master Mix performance in a 4-plex reaction with both DNA and RNA targets.

Manufactured in an ISO 13485–certified facility

TaqPath master mixes are manufactured under a ISO 13485 quality management system that utilizes traceable-quality raw materials and validated operating procedures. The manufacturing of TaqPath products is designed to deliver consistent performance lot after lot.

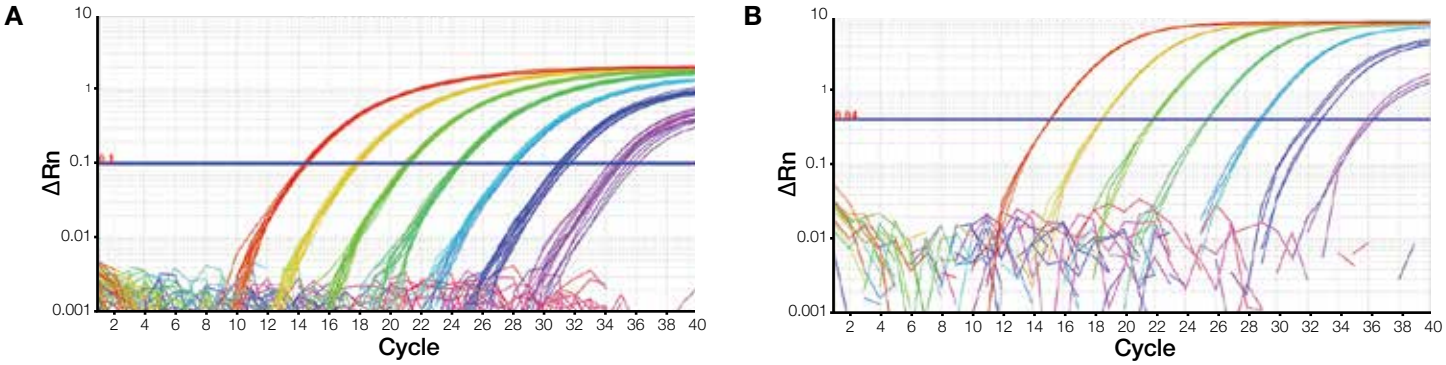


Figure 11. Excellent dynamic range of TaqPath 1-Step RT-qPCR Master Mix. (A) Amplification plots from real-time PCR for a dilution series of poliovirus RNA amplified using the ViiA 7 Real-Time PCR System** and a poliovirus assay (R^2 = 1.0). **(B)** Amplification plot for a dilution series of human DNA with a GAPDH target (R^2 = 1.0).

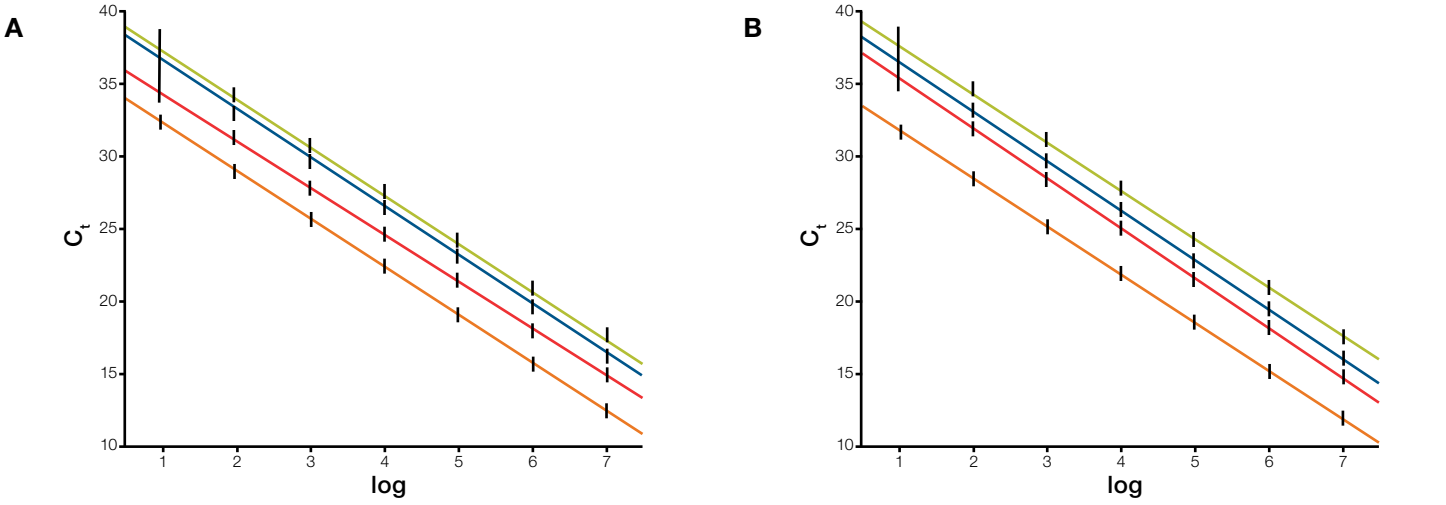


Figure 12. TaqPath 1-Step Multiplex Master Mix is optimized for multiplexing with both RNA and DNA targets. (A) Amplification results for a 4-plex reaction using human cDNA over 6 orders of magnitude, TaqPath 1-Step Multiplex Master Mix, and assays for CD44 (red), CYC1 (green), TMSB10 (orange), and G6PD (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.

General purpose reagents

The TaqPath master mixes are labeled "For Laboratory Use." We are committed to delivering the highest-quality products, service, and support to our customers. TaqPath 1-Step RT-qPCR Master Mix has been tested in an internal benchmarking study against similar master mixes from other suppliers and demonstrated equivalent or better sensitivity and dynamic range across a variety of targets (Table 2).

Table 2. Dynamic range comparison between TaqPath 1-Step RT-qPCR Master Mix and master mixes from other leading suppliers. Comparison of detection range (expressed as number of 10-fold dilutions that yield a standard curve) for a panel of various assays is shown. The criteria for detection were a PCR efficiency between 85% and 115%, and R^2 values >0.98. Each master mix was tested using human RNA and used according to suppliers' respective recommended protocols. Reactions (2–4 replicates) were used on the ViiA 7 Real-Time PCR System.**

Assay	TaqPath 1-step mix	Supplier R	Supplier Q
ANP32B	4	3	4
APOA1	3	4	3
GAPDH	7	7	6
GPX4	6	2	5
TXNDC1	5	5	4
RPLPO (in triplex)	6	5	5
TFRC (in triplex)	4	4	3

Inhibitor tolerant

Unlike other master mixes on the market, the formulation of TaqPath 1-Step Master Mix allows robust performance even in the presence of substances that normally inhibit PCR, such as heparin or hematin, thereby increasing your confidence when working with a variety of complex clinical samples. Figure 13 depicts the enhanced performance of TaqPath 1-Step Multiplex Master Mix in the presence of two common inhibitors as compared to three other suppliers' 1-step kits.

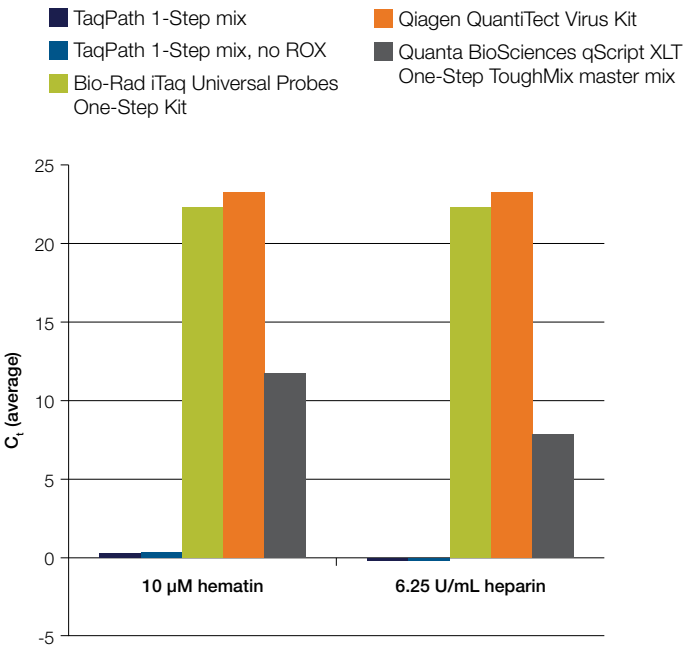


Figure 13. Comparison of inhibitor tolerance of TaqPath 1-Step Multiplex Master Mix and kits from other suppliers. Two inhibitors (hematin and heparin) were added to qRT-PCR reactions run on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System to assess the magnitude of C_t shift caused by these inhibitors. C_t values for reactions without and with inhibitors are shown. The TaqPath 1-Step Multiplex Master Mix includes Mustang Purple dye as a passive reference.

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Ordering information

Product	Quantity	Number of reactions (20 µL)	Cat. No.
TaqPath ProAmp Master Mix (genotyping and CNV)	1 x 1 mL	200	A30865
	1 x 10 mL	2,000	A30866
	2 x 10 mL	4,000	A30871
	1 x 50 mL	5,000	A30867
	2 x 50 mL	10,000	A30872
TaqPath ProAmp Multiplex Master Mix (genotyping and CNV, multiplexing)	1 x 1 mL	200	A30868
	1 x 10 mL	2,000	A30869
	2 x 10 mL	4,000	A30873
	1 x 50 mL	5,000	A30870
	2 x 50 mL	10,000	A30874
TaqPath qPCR Master Mix, CG (gene expression)	1 x 5 mL	500	A15297
	2 x 5 mL	1,000	A16245
	5 x 5 mL	2,000	A16247
	10 x 5 mL	5,000	A16248
	1 x 5 mL	5,000	A15298
TaqPath 1-Step RT-qPCR Master Mix, CG (gene expression, one-step)	1 x 5 mL	1,000	A15299
	1 x 10 mL	2,000	A15300
TaqPath 1-Step Multiplex Master Mix (gene expression, one-step multiplex)	1 x 0.5 mL	100	A28525
	5 x 1 mL	1,000	A28526
	1 x 10 mL	2,000	A28527
TaqPath 1-Step Multiplex Master Mix (No ROX) (gene expression, one-step multiplex)	1 x 0.5 mL	100	A28521
	5 x 1 mL	1,000	A28522
	1 x 10 mL	2,000	A28523

* 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.
** TaqMan assays and the ViiA 7 Real-Time PCR System are For Research Use Only. Not for use in diagnostic procedures.

Find out more at **thermofisher.com/taqpath**

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