Confidence in clinical testing: TaqPath general purpose reagents

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 with stringent production and process controls to help ensure lot-to-lot consistency
- Labeled "For Laboratory Use"

TaqPath general purpose reagent	Application	Passive reference dye
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 1-Step RT-qPCR Master Mix

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

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 2 shows consistent C_t results obtained from three distinct lots when detecting 10-copy inputs of RNA target. Figure 3 demonstrates that lot-to-lot consistency of C_t is preserved across multiple assays— with different attributes and detection of different levels of expression.

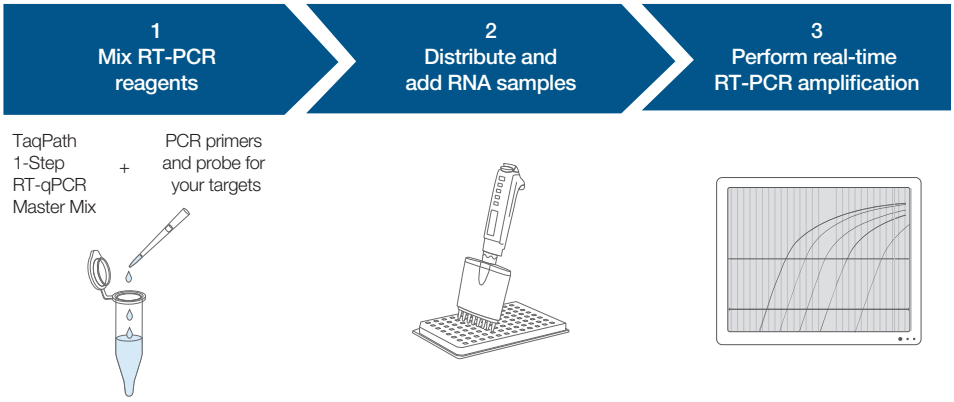


Figure 1. The simple TaqPath 1-Step RT-qPCR Master Mix workflow.

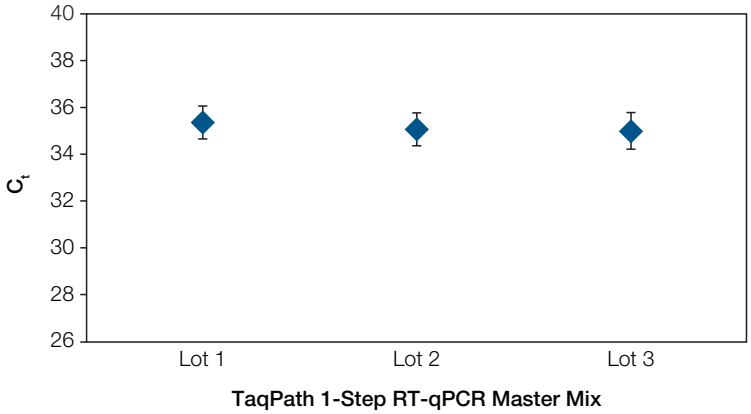


Figure 2. Reliable low-copy detection. Samples containing 10 copies of RNA target were amplified using three distinct TaqPath 1-Step RT-qPCR Master Mix lots and an RNase P assay.

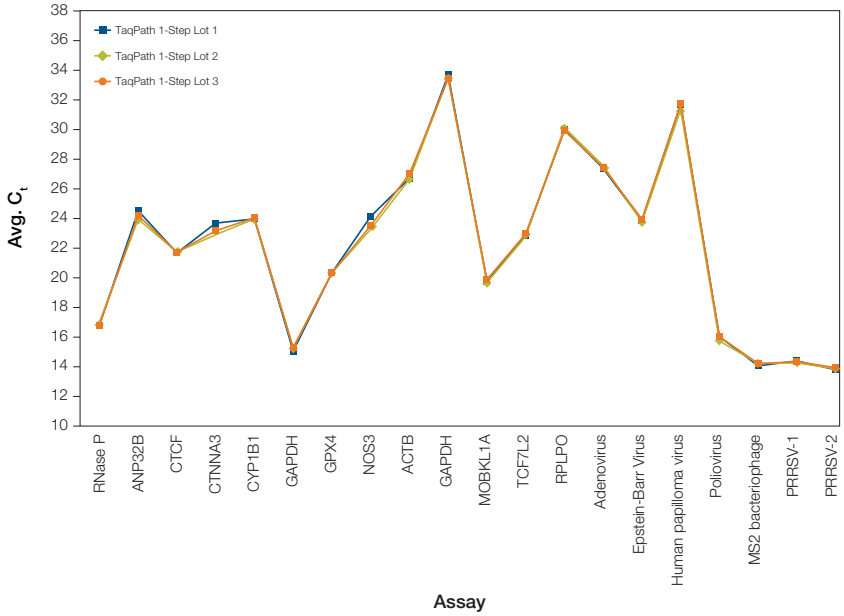


Figure 3. C_t consistency across multiple assays for three unique lots of TaqPath 1-Step RT-qPCR Master Mix. Total RNA was amplified using a panel of human and viral gene expression assays and three distinct lots of TaqPath 1-Step RT-qPCR Master Mix. Excellent C_t concordance is seen across the three 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 4 demonstrates the 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 5 demonstrates TaqPath 1-Step Multiplex Master Mix performance in a 4-plex reaction with both DNA and RNA targets.

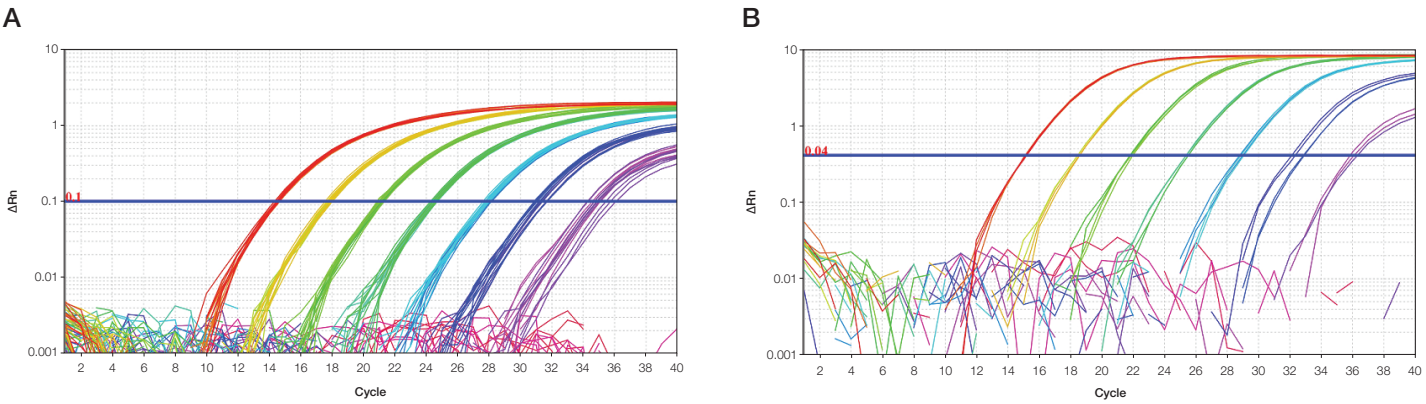


Figure 4. Excellent dynamic range of TaqPath 1-Step RT-qPCR Master Mix. (A) Amplification plots from real-time PCR for a dilution series of poliovirus RNA amplified using the Applied Biosystems™ ViiA™ 7 Real-Time PCR System† and a poliovirus assay (R^2 = 1.0). **(B)** Amplification plot for a dilution series of human DNA with a GAPDH target (R^2 = 1.0).

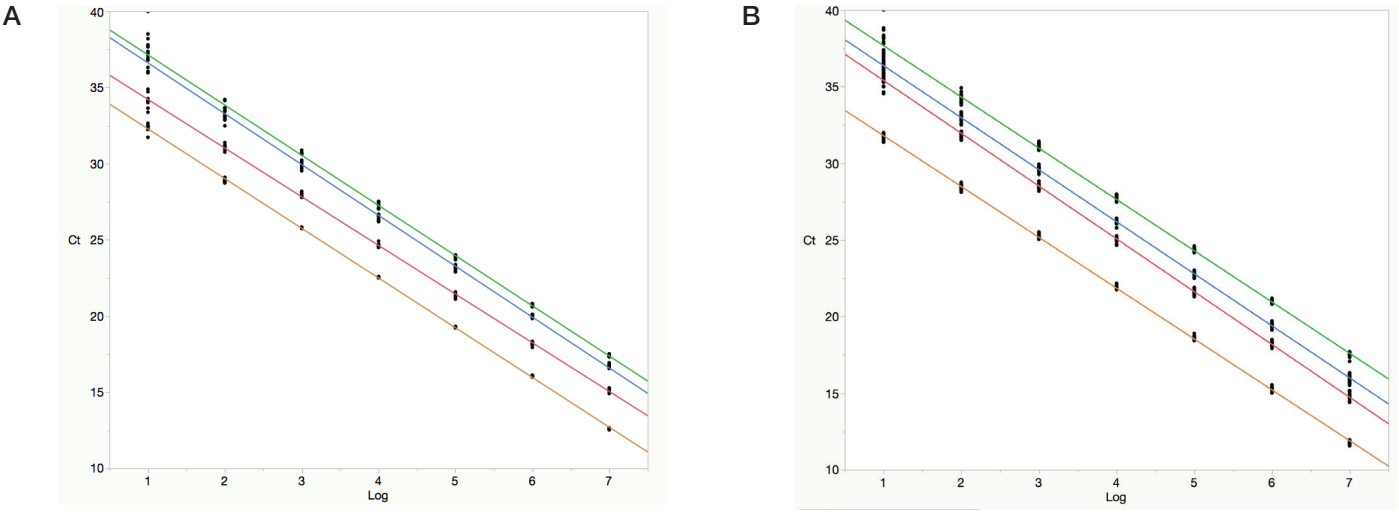


Figure 5. TaqPath 1-Step Multiplex Master Mix is optimized for multiplexing with both RNA and DNA targets. (A) Amplification results for a 4-plex reaction using human cDNA over 6 orders of magnitude, TaqPath 1-Step Multiplex Master Mix, and assays for CD44 (red), CYC1 (green), TMSB10 (orange), and G6PD (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.

TaqPath 1-Step RT-qPCR Master Mix has been tested in an internal benchmarking study against similar competitor master mixes and demonstrated equivalent or better sensitivity and dynamic range across a variety of targets (Table 1).

Inhibitor tolerant

Unlike other master mixes on the market, the formulation of TaqPath 1-Step Master Mixes allows robust performance even in the presence of substances that normally inhibit PCR, such as heparin or hematin, increasing your confidence when working with a variety of complex clinical samples. Figure 6 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.

TaqPath qPCR Master Mix, CG

Applied Biosystems™ TaqPath™ qPCR Master Mix, CG is designed, with all lots functionally tested, to help ensure lot-to-lot reproducibility for C_t consistency and dynamic range across a wide variety of assays. With stringent

Table 1. Dynamic range comparison between TaqPath 1-Step RT-qPCR Master Mix and mixes from other leading suppliers. Comparison of detection range (expressed as number of detected 10-fold dilutions) for a panel of various assays. 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 manufacturers' respective recommended protocols. Reactions (2–4 replicates) were used on the ViiA 7 Real-Time PCR System.[1]

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

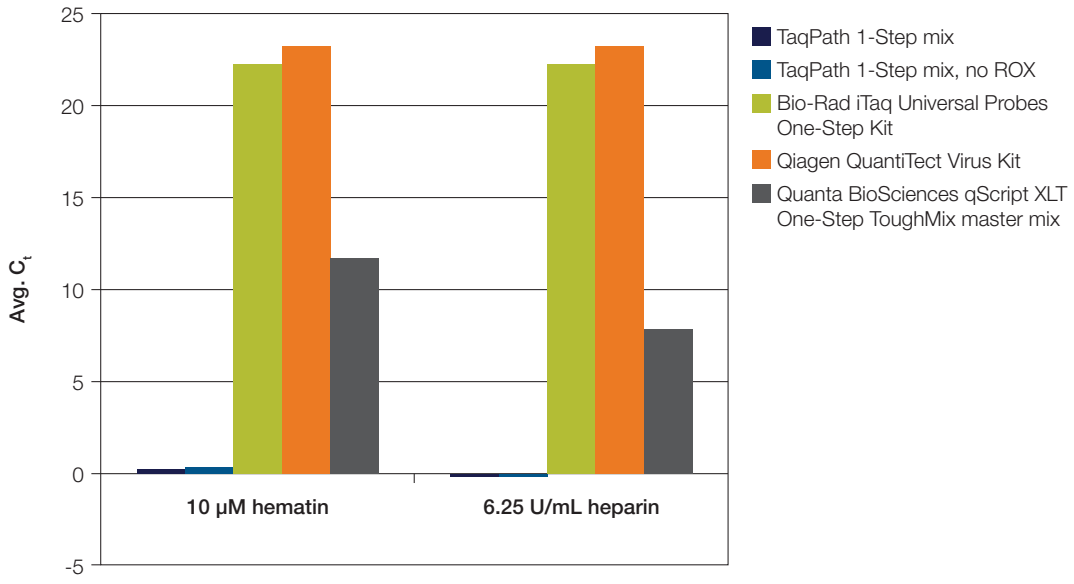


Figure 6. Comparison of inhibitor tolerance of TaqPath 1-Step Multiplex Master Mix and kits from other suppliers. Two inhibitors (hematin and heparin) were added to RT-qPCR reactions run on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System to assess the magnitude of C_t shift caused by these inhibitors. C_t values for reactions without and with inhibitors are shown. The TaqPath 1-Step Multiplex Master Mix includes MUSTANG PURPLE dye as a passive reference.

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 across up to 7 orders of magnitude with gene expression or miRNA assays*
- Enables reliable detection of low-copy templates with reproducible C_t results
- 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, 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 7 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 8). TaqPath qPCR Master Mix, CG was tested in an internal

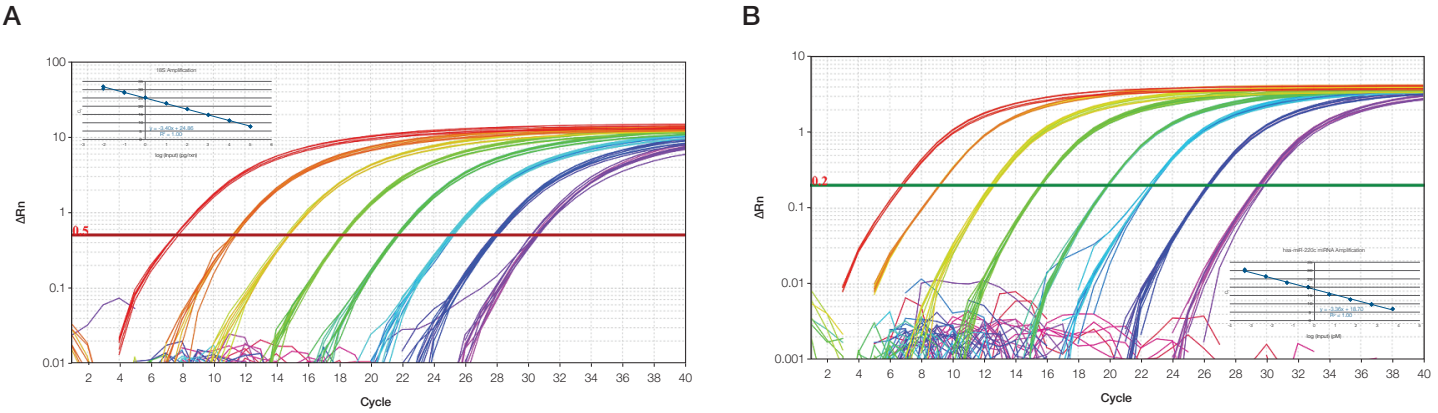
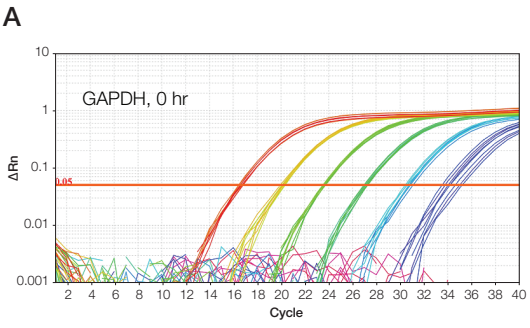
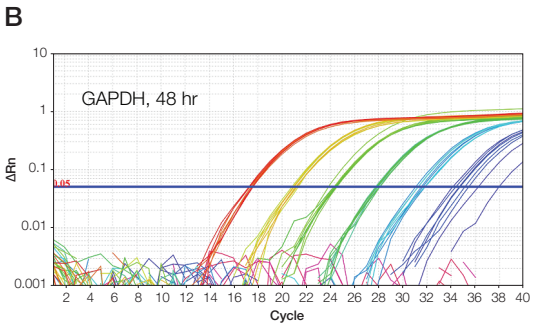


Figure 7. Excellent dynamic range of TaqPath qPCR Master Mix, CG. Representative amplification plots using the ViiA 7 Real-Time PCR System† and **(A)** an 18S assay with human cDNA dilution series or **(B)** an hsa-miR-220c miRNA assay with an artificial template dilution series.





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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 8. Benchtop stability of TaqPath qPCR Master Mix, CG. The GAPDH gene expression assay was run upon assembly, or **(A)** 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, comparable to time 0.

benchmarking study against similar competitor master mixes and demonstrated equivalent or better sensitivity and dynamic range across a variety of targets (Table 2).

Reproducible, sensitive detection

We understand the importance of reliably 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 9 shows the consistent C_t results obtained from three distinct lots when detecting 10-copy inputs. This lot-to-lot C_t consistency is preserved across multiple assays with different attributes and detection of different levels of expression to maximize confidence in your results (Figure 10).

Table 2. Dynamic range comparison between TaqPath qPCR Master Mix, CG and other leading suppliers. Comparison of detection range (expressed as number of detected 10-fold dilutions) for a panel of various assays. 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 manufacturers' 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

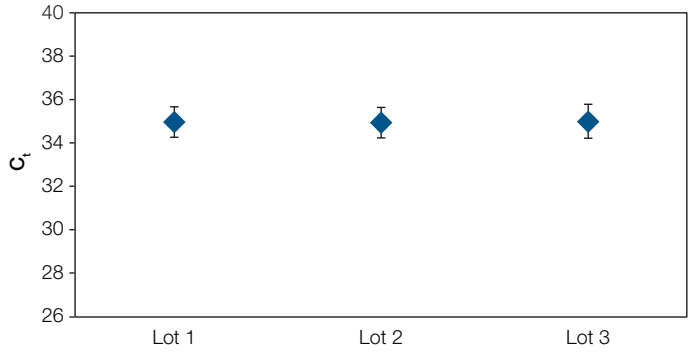

Figure 9. Reliable low-copy detection. A sample containing 10 copies of human DNA was amplified using three distinct TaqPath qPCR Master Mix, CG lots and an RNase P assay.

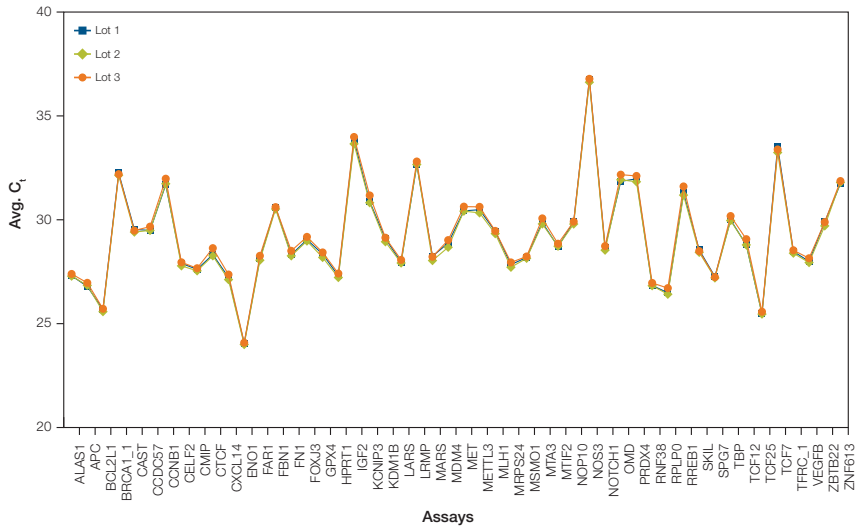

Figure 10. C_t consistency across multiple assays for three lots of TaqPath qPCR Master Mix, CG. Human cDNA was amplified using a panel of 96 gene expression assays and three distinct lots of TaqPath qPCR Master Mix, CG. Excellent C_t concordance is seen across the three lots for a representative subset 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 duplex reaction performance in each manufacturing lot (Figure 11).

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, 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 12).

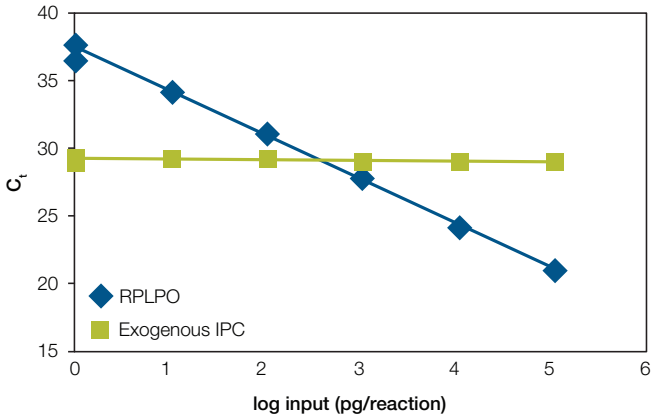

Figure 11. TaqPath qPCR Master Mix, CG is optimized for multiplexing. Amplification results are shown for a duplex reaction using human cDNA and an RPLPO (large ribosomal protein) assay with an exogenous internal positive control (IPC).

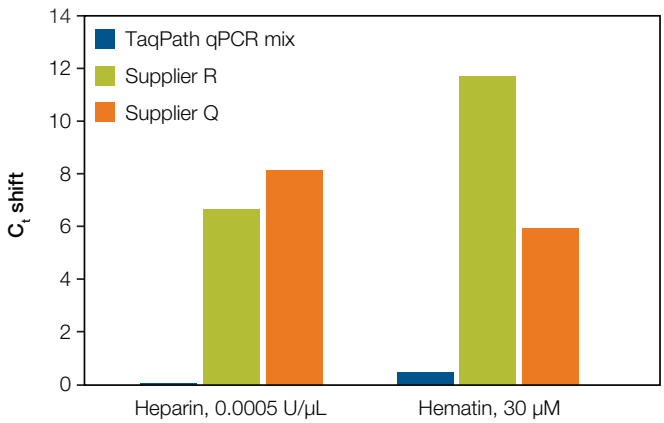

Figure 12. Comparison of inhibitor tolerance of TaqPath qPCR Master Mix, CG and kits from other suppliers. Two inhibitors of qPCR, heparin and hematin, were added to RT-qPCR reactions to assess the magnitude of C_t shift caused by these inhibitors. Graphs show the change in C_t from the baseline value with no inhibitor present.

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Manufacturing production and process controls

TaqPath master mixes are manufactured under a quality management system that utilizes traceable-quality raw materials and validated operating procedures. With established controls from purchasing through QC release, the manufacturing of TaqPath™ products is designed to deliver consistent performance lot after lot.

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.

Ordering information

Product	Quantity	Number of reactions (20 µL)	Cat. No.
TaqPath 1-Step RT-qPCR Master Mix, CG	5 x 1 mL	1,000	A15299
	1 x 10 mL	2,000	A15300
TaqPath 1-Step Multiplex Master Mix	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)	1 x 0.5 mL	100	A28521
	5 x 1 mL	1,000	A28522
	1 x 10 mL	2,000	A28523
TaqPath qPCR Master Mix, CG	1 x 5 mL	500	A15297
	2 x 5 mL	1,000	A16245
	5 x 5 mL	2,500	A16247
	10 x 5 mL	5,000	A16248
	1 x 50 mL	5,000	A15298

* 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.
† The ViiA 7 Real-Time PCR System is For Research Use Only. Not for use in diagnostic procedures.

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

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