TaqPath ProAmp Master Mixes

Applied Biosystems™ TaqPath™ ProAmp™ Master Mixes are versatile master mixes developed for high-throughput genotyping and copy number variation (CNV) analysis protocols that require accurate results from samples containing PCR inhibitors. TaqPath ProAmp Master Mixes are designed to deliver sensitive and reproducible results from genomic DNA targets. The simple genotyping and CNV analysis workflow (Figure 1) and reproducible performance even in the presence of inhibitors offer confidence in results.

We offer two TaqPath ProAmp Master Mix formulations for different throughput needs:

- TaqPath ProAmp Master Mix, which includes ROX™ passive reference dye
- TaqPath ProAmp Multiplex Master Mix, which includes Mustang Purple™ passive reference dye for multiplexing up to 4 targets

Both mixes enable measurement of FAM™, VIC™, ABY™, and 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 single nucleotide polymorphism (SNP) genotyping and other higher-multiplex applications.

These two formulations expand our line of Applied Biosystems™ TaqPath™ general purpose reagents to include master mixes for genotyping and CNV analysis in addition to qPCR and RT-qPCR master mixes, all of which are manufactured in an ISO 13485–certified facility with stringent production and process controls to help ensure excellent manufacturing consistency. With over 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.

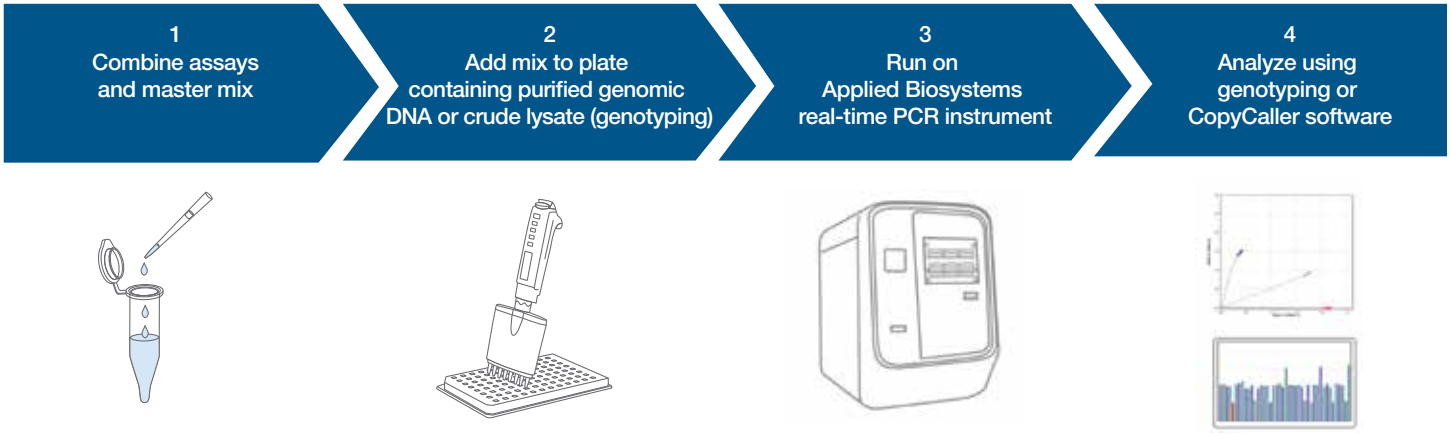

Figure 1. Workflow for TaqPath ProAmp Master Mix.

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Features of TaqPath ProAmp Master Mix

- **Exceptional data quality**—high specificity, dynamic range,* and reproducibility for genotyping and copy number determination, even in the presence of inhibitors
- **Tolerance to inhibitors**—compatible with samples prepared from human or animal sources (buccal swabs, blood, and card punches)
- **72-hour pre-PCR benchtop stability**—flexible plate setup for automated workflows, enabled by our Dual-Lock™ *Taq* DNA polymerase's hot-start mechanism
- **Multiplexing breadth**—two formulations that enable detection of up to four targets per reaction
- **Excellent manufacturing consistency**—manufactured in an ISO 13485–certified facility to help ensure excellent manufacturing consistency

High-accuracy genotyping

Accuracy in genotyping is important where limited amounts of samples are available, for example in clinical diagnostic testing. TaqPath ProAmp Master Mix has been optimized to yield accurate and reproducible results, even in low-volume reactions, with 1 ng input per reaction. Figure 2 shows a comparison of genotyping accuracy between TaqPath ProAmp Master Mix and other commercial master mixes for the same set of Applied Biosystems™ TaqMan® Drug Metabolism SNP Genotyping Assays** using crude lysate from blood samples. TaqPath ProAmp Master Mix has been optimized to provide high specificity for allelic discrimination, as demonstrated by excellent cluster separation in genotyping plots, whether the sample is purified genomic DNA or crude lysate.

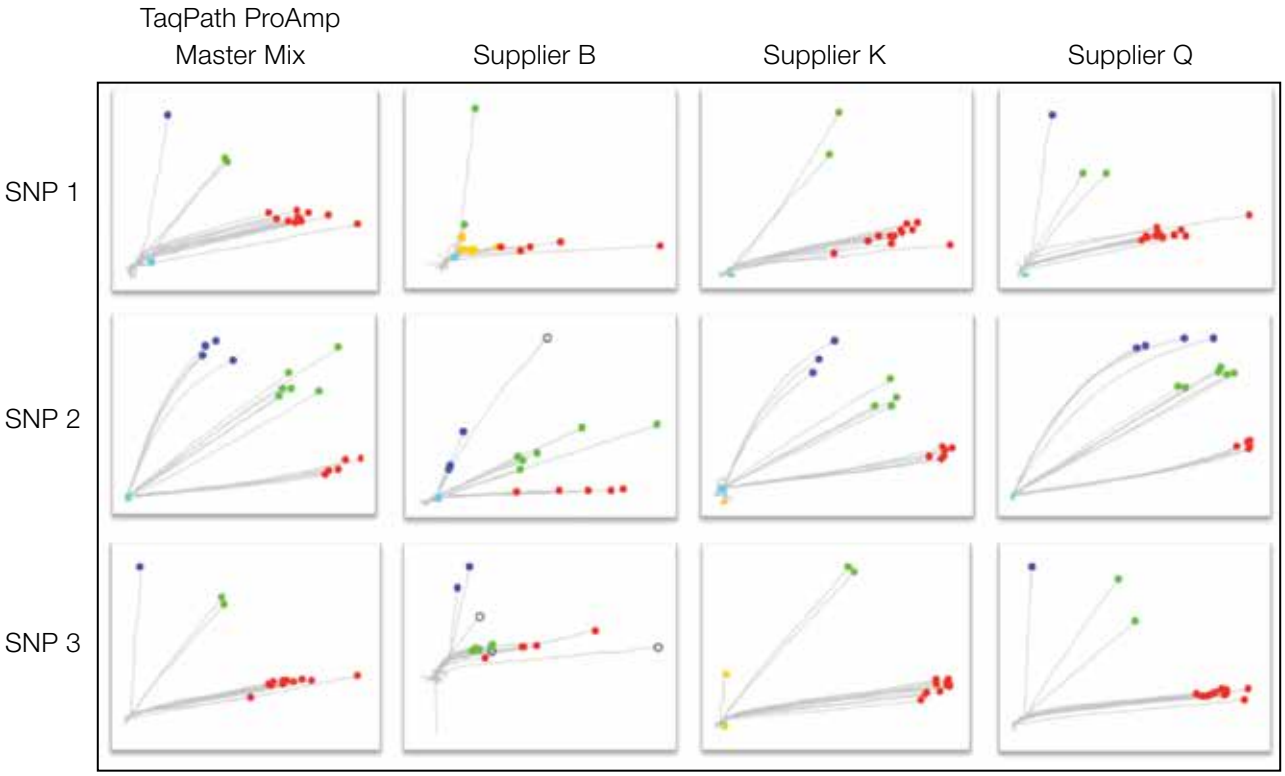

Figure 2. Genotyping results from TaqPath ProAmp Master Mix vs. other commercial master mixes. Crude lysates were prepared from blood samples and diluted 20-fold. Results are shown for three TaqMan Drug Metabolism SNP Genotyping Assays run under standard conditions. TaqPath ProAmp Master Mix consistently produced accurate genotype calls and excellent cluster resolution across multiple assays. Dark blue, green, and red colors correspond to data with genotype calls assigned by the Thermo Fisher Cloud Genotyping application. Light blue corresponds to no template (no DNA) controls, yellow corresponds to samples with signal too low to assign genotype calls, and black corresponds to samples of undetermined genotype.

High-accuracy copy number quantitation

The versatile TaqPath ProAmp Master Mix can also be used for highly accurate CNV analysis using purified genomic DNA samples. Figure 3 shows a comparison of CNV results using TaqPath ProAmp Master Mix and two other commercial master mixes. TaqPath ProAmp Master Mix can accurately determine copy number in samples with up to 4 copies (data not shown).

High reproducibility

Clinical diagnostic testing requires high reproducibility in genotyping and copy number results. TaqPath ProAmp Master Mix has been optimized to achieve low standard deviation in C_t values, enabling highly reproducible results with both fast and standard ramp rates, even with samples containing PCR inhibitors. Figure 4 shows a comparison of C_t standard deviations derived from the use of TaqPath ProAmp Master Mix and three other commercial master mixes with an RNase P gene assay that typically serves as a reference in CNV quantitation. The results demonstrated high reproducibility from TaqPath ProAmp Master Mix.

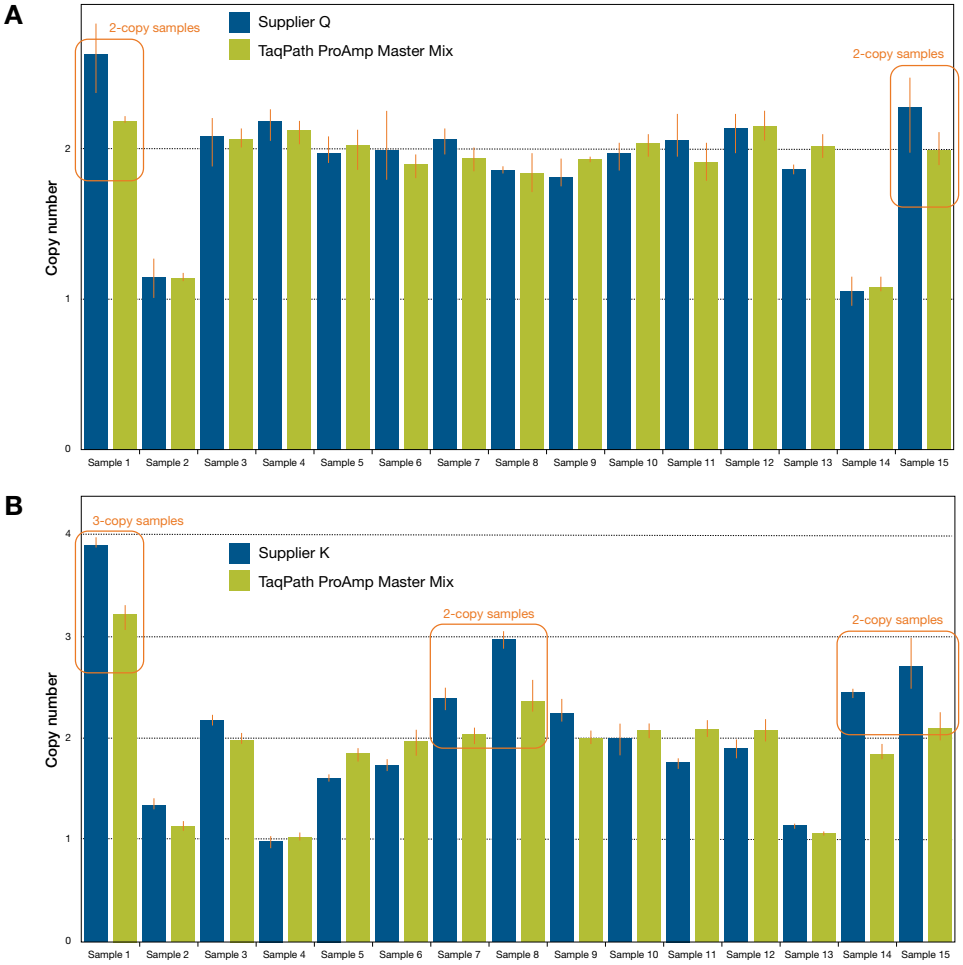

Figure 3. CNV analysis using TaqPath ProAmp Master Mix vs. other commercial master mixes. TaqPath ProAmp Master Mix yields copy number results closer to expected integer values. **(A)** Copy number quantitation results for *CYP2D6* intron 2, based on assays run using TaqPath ProAmp Master Mix (green) and Supplier Q mix (blue). Genomic DNA purified from buccal swabs using the Applied Biosystems™ MagMAX™ DNA Multi-Sample Kit was used at an input of 10 ng per well, 4 replicates per sample. **(B)** Copy number quantitation results for *CYP2D6* intron 6, based on assays run using TaqPath ProAmp Master Mix (green) and Supplier K mix (blue). Genomic DNA from blood was purified and used as described in (A). Annotations in orange ("2-copy samples", "3-copy samples") highlight samples that demonstrate the superior accuracy and reliability of TaqPath ProAmp Master Mix, in comparison to the alternative supplier's product.

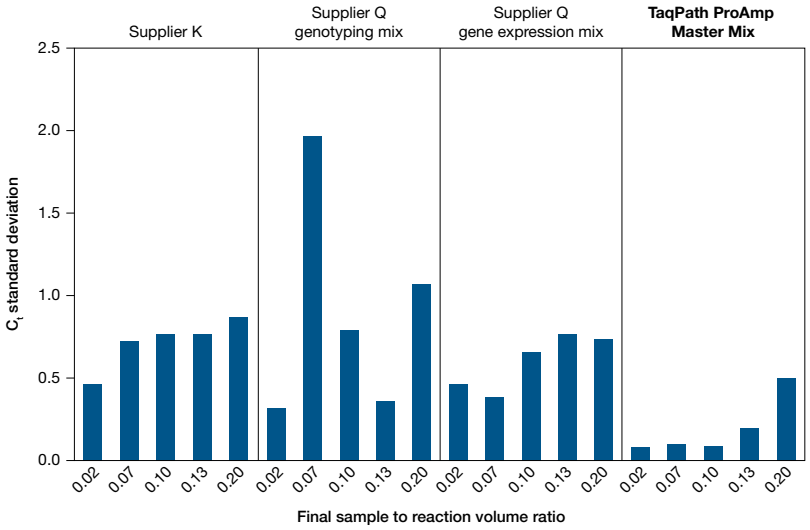

Figure 4. Mean C_t standard deviations for TaqPath ProAmp Master Mix vs. other commercial master mixes. Results are based on 8 replicate qPCR reactions using an RNase P gene assay and increasing ratios of blood sample volume to reaction volume. TaqPath ProAmp Master Mix has a low standard deviation for 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.

Multiplexing

Both TaqPath ProAmp Master Mix and TaqPath ProAmp Multiplex Master Mix can be used for multiplex reactions, allowing additional exogenous or endogenous controls or targets to be assayed 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, and ABY reporter dye labels and QSY™ quenchers to provide detection of 3 targets and ROX dye as a passive reference in a single reaction. Figure 5 demonstrates the performance of TaqPath ProAmp Master Mix in a 3-plex experiment using DNA from dried blood on filter paper to detect 3 targets simultaneously: T cell receptor excision circles (TREC) with FAM dye, kappa-deleting recombination excision circles (KREC) with VIC dye, and the endogenous control gene *RPPH1* (RNaseP) labeled with ABY dye.

TaqPath ProAmp Multiplex Master Mix also allows use of a fourth probe with JUN dye and Mustang Purple dye as a passive reference dye. These dyes are optimized to work together—in both formulations of the master mix—with minimal spectral overlap for optimal performance.

Inhibitor tolerance

Unlike other master mixes on the market, the unique proprietary formulation of TaqPath ProAmp Master Mix enables reproducibly accurate performance even in the presence of substances that normally inhibit PCR, such as heparin, EDTA, and sodium citrate, increasing your confidence when working with a variety of complex clinical samples. Figure 6 depicts the enhanced performance of TaqPath ProAmp Master Mix in the presence of two common inhibitors, in comparison with two other commercial master mixes.

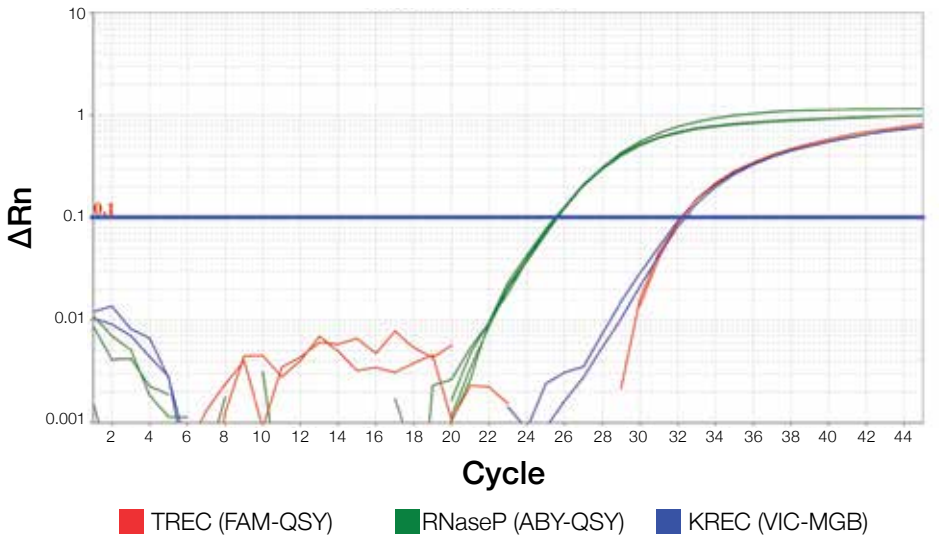

Figure 5. Multiplex PCR. Applied Biosystems™ TaqMan® Gene Expression Assays** were used in a 3-plex experiment targeting TREC with a FAM–QSY probe, KREC with a VIC–MGB/NFQ probe, and RNaseP (endogenous control gene) with an ABY–QSY probe. The Applied Biosystems™ DNA Extract All Reagents Kit was used to extract DNA in 3 minutes from a 2 mm filter paper containing dried blood. A 5 µL volume of stabilized solution prepared with the DNA Extract All Reagents Kit was used in a 20 µL qPCR reaction with TaqPath ProAmp Master Mix.

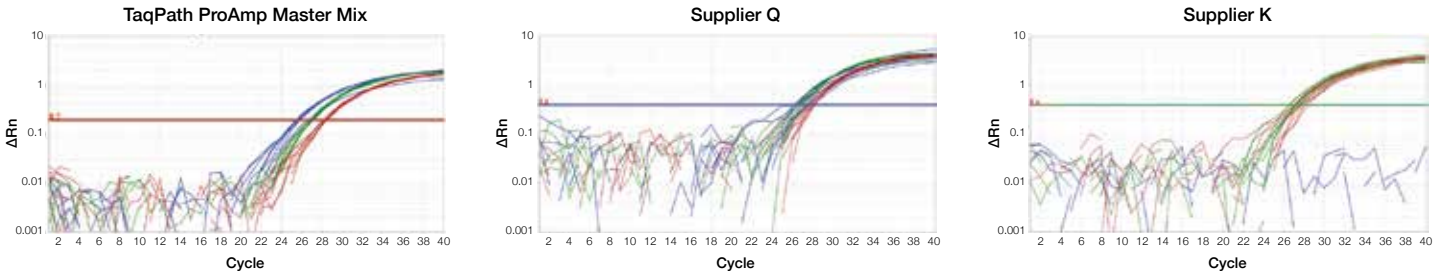

Figure 6. Inhibitor tolerance of TaqPath ProAmp Master Mix and other commercial master mixes. Crude lysates of blood samples isolated in sodium citrate (green), EDTA (red), or heparin (blue) Vacutainer™ tubes were used in the experiment and constituted 30% of reaction volume. Eight replicate genotyping reactions were run to assess the magnitude and standard deviation of C_t values in the presence of these PCR inhibitors. Amplification plots for reactions are shown. TaqPath ProAmp Master Mix yields lower C_t values in the presence of inhibitors, and lower standard deviation for replicates. No amplification was observed using Supplier K's mix with the heparin-containing sample.

Manufacturing production and process controls

With established controls from purchasing through QC release, the manufacturing of TaqPath™ products is designed to deliver consistent performance across multiple lots. Figure 7 demonstrates that lot-to-lot consistency of C_t is preserved across multiple samples at the same target levels.

Broad instrument compatibility

TaqPath ProAmp Master Mix can be used in either fast or standard cycling conditions with equivalent performance across a wide variety of real-time PCR systems, including Applied Biosystems™ qPCR platforms (e.g., 7500, 7500 Fast, and 7500 Fast Dx systems; QuantStudio™ 3, 5, 6 Flex, 7 Flex, 12K Flex, and Dx systems; ViiA™ 7 system).**

General purpose reagents

TaqPath master mixes are general purpose reagents manufactured in an ISO 13485–certified facility and labeled "For Laboratory Use". TaqPath master mixes are part of the TaqPath general purpose reagents product line, which includes:

- **TaqPath™ qPCR Master Mix, CG**— includes ROX passive reference dye
- **TaqPath™ 1-Step RT-qPCR Master Mix, CG**— 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
- **TaqPath™ ProAmp Master Mix**— includes ROX passive reference dye
- **TaqPath™ ProAmp Multiplex Master Mix**— includes Mustang Purple passive reference dye

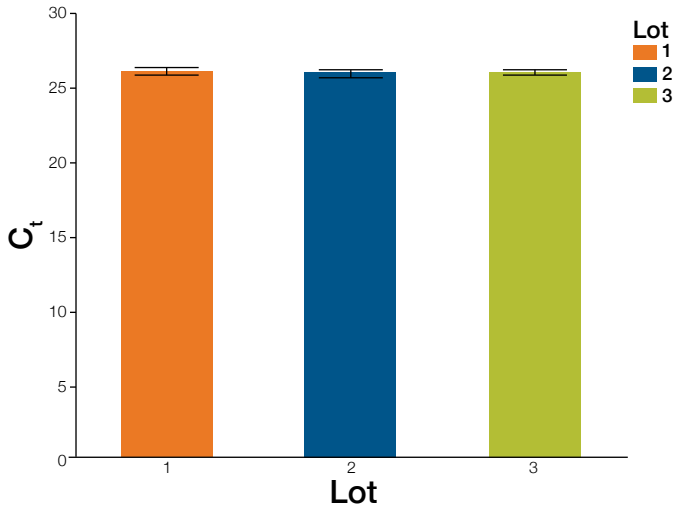

Figure 7. Consistency of C_t across multiple assays for three unique lots of TaqPath ProAmp Master Mix. Genomic DNA was analyzed using the Applied Biosystems™ TaqMan® Copy Number Reference Assay** for RNase P and three distinct lots of TaqPath ProAmp Master Mix on a panel of 23 DNA samples. Mean C_t values for each lot are shown with error bars representing one standard deviation; the standard deviations for lots 1, 2, and 3 were 0.199, 0.255, and 0.278, respectively. Excellent C_t concordance was seen across the three lots, and copy number values were 100% concordant across all lots tested with 20 assays, 23 samples, and 4 replicates per assay/sample combination.

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Ordering information

Product	Reactions	Quantity	Cat. No.
TaqPath ProAmp Master Mix	200	1 x 1 mL	A30865
	2,000	1 x 10 mL	A30866
	4,000	2 x 10 mL	A30871
	10,000	1 x 50 mL	A30867
	20,000	2 x 50 mL	A30872
TaqPath ProAmp Master Mix, evaluation samples	200	1 x 1 mL	A32704
TaqPath ProAmp Multiplex Master Mix	200	1 x 1 mL	A30868
	2,000	1 x 10 mL	A30869
	4,000	2 x 10 mL	A30873
	10,000	1 x 50 mL	A30870
	20,000	2 x 50 mL	A30874
TaqPath ProAmp Multiplex Master Mix, evaluation samples	200	1 x 1 mL	A32705

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

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