PrimeTime™ ONE-STEP 4X BROAD-RANGE MASTER MIX

For probe-based qPCR assays

Flexibility to amplify from purified or crude samples

More data confidence with higher endpoint fluorescence

Inhibitor-resistant

PREMIUM ONE-STEP RT-qPCR MASTER MIX FOR VIRAL RESEARCH AND GENE EXPRESSION

PrimeTime One-Step 4X Broad-Range Master Mix is a ready-to-use, 4X concentrated mixture of hot-start reverse transcriptase, hot-start DNA polymerase, dNTPs, $MgCl_2$, enhancers and stabilizers. This Master Mix provides optimal performance for analysis of RNA in probe-based qPCR assays. The optional enhancer solution also can be used to neutralize PCR inhibitors and nucleases during crude sample amplification.

AMPLIFY FROM PURIFIED OR CRUDE SAMPLES

PrimeTime One-Step 4X Broad-Range Master Mix has been specifically designed to give you the option of amplifying from purified RNA samples or direct amplification from viral transport media - VTM (universal transport media - UTM). The optional enhancer solution is formulated to neutralize PCR inhibitors that are commonly present in crude samples. Human saliva (Figure 1) and nasopharyngeal (Figure 2) samples were tested. Amplifying directly from crude samples removes the need for sample prep, which simplifies your workflow.

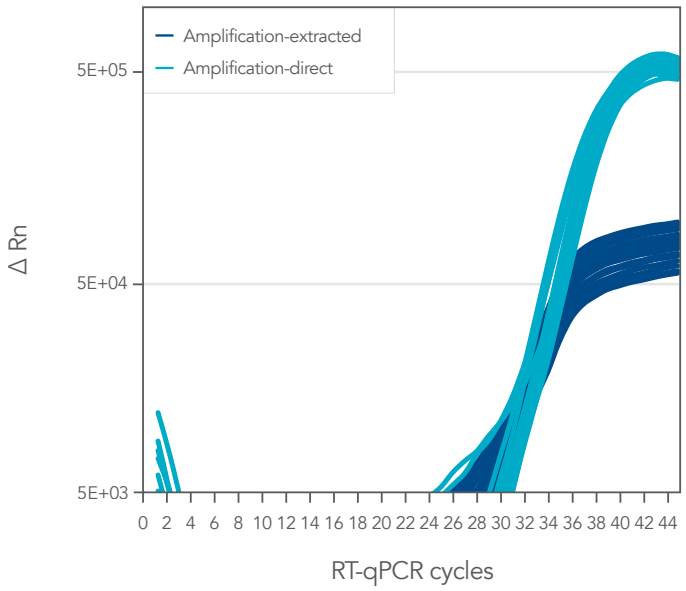

Figure 1. PrimeTime One-Step 4X Broad-Range Master Mix demonstrates comparable amplification of viral analytes between extracted and non-extracted human saliva samples. RT-qPCR assays containing probes and primers specific for FluA, FluB, SARS-CoV-2, and RNase P were used to amplify 30 copies of AccuPlex™ SARS-CoV-2, Flu A/B and RSV Verification Panel (SARS-CoV-2 data shown) material that had been extracted (dark blue) or direct amplified (light blue). The AccuPlex analyte verification panel was first diluted into negative human saliva matrix prior to RNA extraction or direct specimen amplification, and RNA extractions were automated using the KingFisher™ Flex 96 and MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit. Reactions were amplified and identified using the QuantStudio™ 7 Flex instrument according to the manufacturer's instructions. Experiments were performed with multiple replicates (n = 20) for each condition.

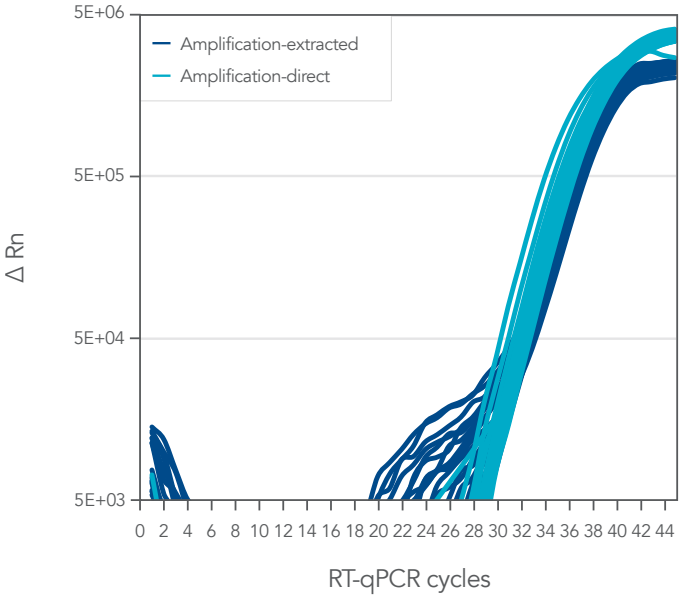

Figure 2. PrimeTime One-Step 4X Broad-Range Master Mix demonstrates comparable amplification of viral analytes between extracted and non-extracted human nasopharyngeal samples. RT-qPCR assays containing probes and primers specific for FluA, FluB, SARS-CoV-2, and RNase P were used to amplify 30 copies of AccuPlex SARS-CoV-2, Flu A/B and RSV Verification Panel (SARS-CoV-2 data shown) material that had been extracted (dark blue) or direct amplified (light blue). The AccuPlex analyte verification panel was first diluted into negative human nasopharyngeal (NP) matrix prior to RNA extraction or direct specimen amplification, and RNA extractions were performed automated using the KingFisher Flex 96 and MagMAX Viral/ Pathogen II (MVP II) Nucleic Acid Isolation Kit. Reactions were amplified and detected using the QuantStudio 7 Flex instrument according to the manufacturer's instructions. Experiments were performed with multiple replicates (*n* = 20) for each condition.

HIGH ENDPOINT FLUORESCENCE AND LOW C_t

Particularly at later C_t values, many qPCR assays tend to have reduced relative fluorescent units (RFU). This can make it difficult to analyze these later amplification curves. The enzymes used in the PrimeTime One-Step 4X Broad-Range Master Mix have been developed to maintain high activity even in later qPCR cycles. The optimized enzymes and buffers result in high endpoint fluorescence and often earlier C_t values as compared to other commercially available qPCR master mixes (Figure 3).

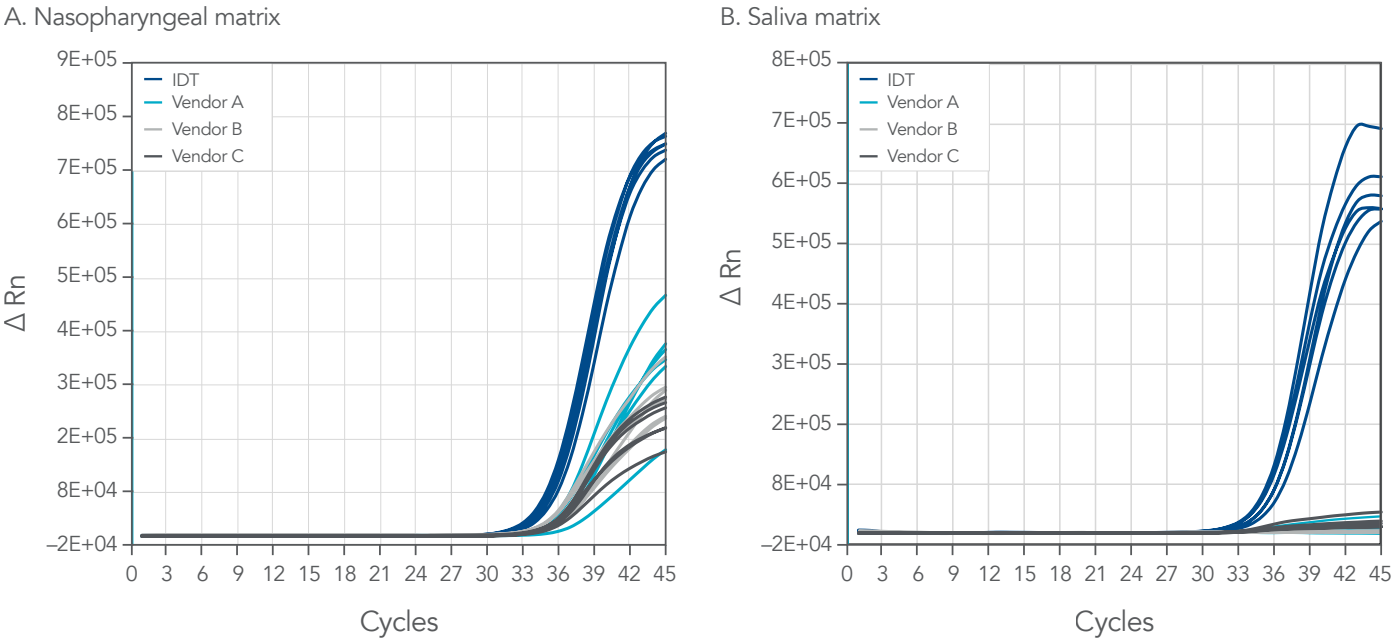

Figure 3. PrimeTime One-Step 4X Broad-Range Master Mix provides higher endpoint fluorescence values from human saliva and nasopharyngeal matrix as compared to competitors. RT-qPCR assays containing probes and primers specific for Influenza A (shown), Influenza B, SARS-CoV-2, and RNaseP genes were used to amplify 30 copies of pooled viral analytes spiked into negative human nasopharyngeal (left) or saliva (right) matrix stored in viral transport medium. RT-qPCR reactions were run on the QuantStudio 7 Flex (*n* = 6).

INHIBITOR RESISTANT

Samples that contain PCR inhibitors can cause problems in amplification and data analysis. The PrimeTime One-Step 4X Broad-Range Master Mix is designed to handle these types of samples and give consistent amplification results. The data below shows amplification of samples with common PCR inhibitors heparin (Figure 4), hematin (Figure 5), humic acid (Figure 6) spiked-in to reactions.

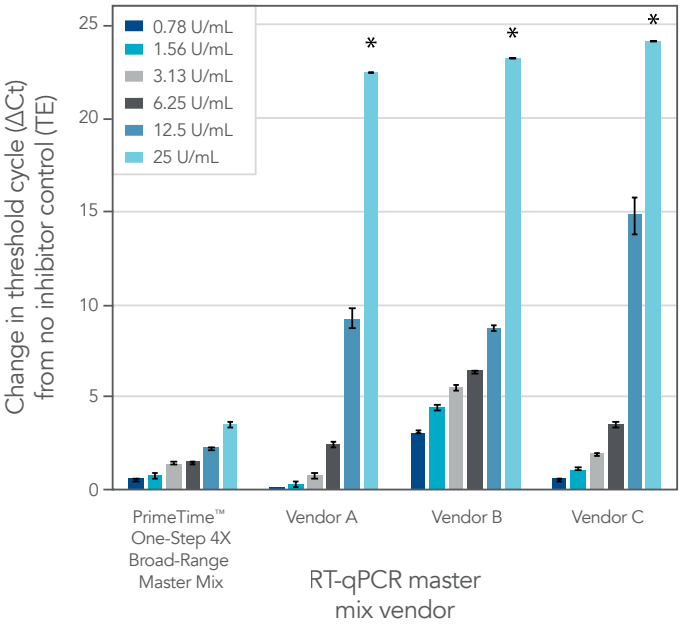

Figure 4. PrimeTime One-Step 4X Broad-Range Master Mix has a higher tolerance to heparin inhibition than three other commercially available master mixes. RT-qPCR probe assays containing probes and primers specific for Flu A (shown), Flu B, SARS-CoV-2, and RNase P genes were used to amplify RNA template in the presence of heparin concentrations ranging from 0.78–25 U/mL. Each master mix was used according to the manufacturer's instructions. RT-qPCR reactions were run on the QuantStudio 7 Flex (*n* = 6, per inhibitor concentration per master mix). Error bars represent standard deviation.

* Indicates reactions where no amplification occurred in the presence of heparin.

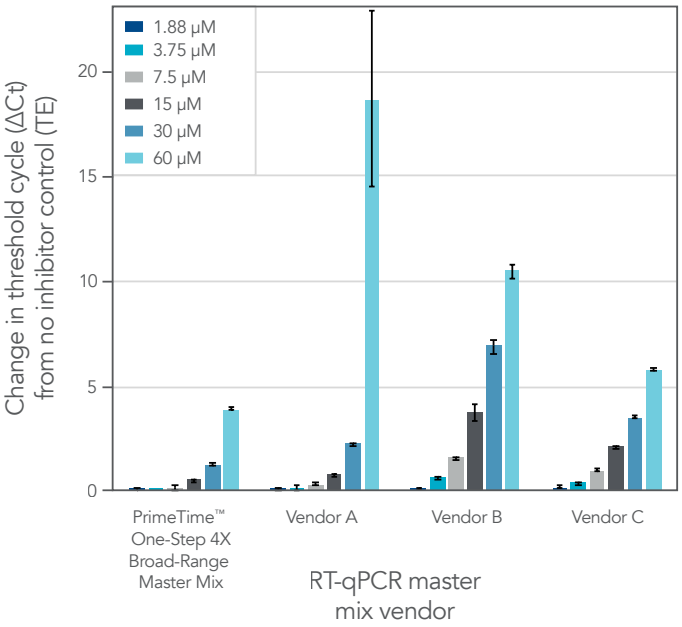

Figure 5. PrimeTime One-Step 4X Broad-Range Master Mix has a higher tolerance to hematin inhibition than three other commercially available master mixes. RT-qPCR probe assays containing probes and primers specific for Flu A (shown), Flu B, SARS-CoV-2, and RNase P genes were used to amplify RNA template in the presence of hematin concentrations ranging from 1.88–60 µM. Each master mix was used according to the manufacturer's instructions. RT-qPCR reactions were run on the QuantStudio 7 Flex (*n* = 6, per inhibitor concentration per master mix). Error bars represent standard deviation.

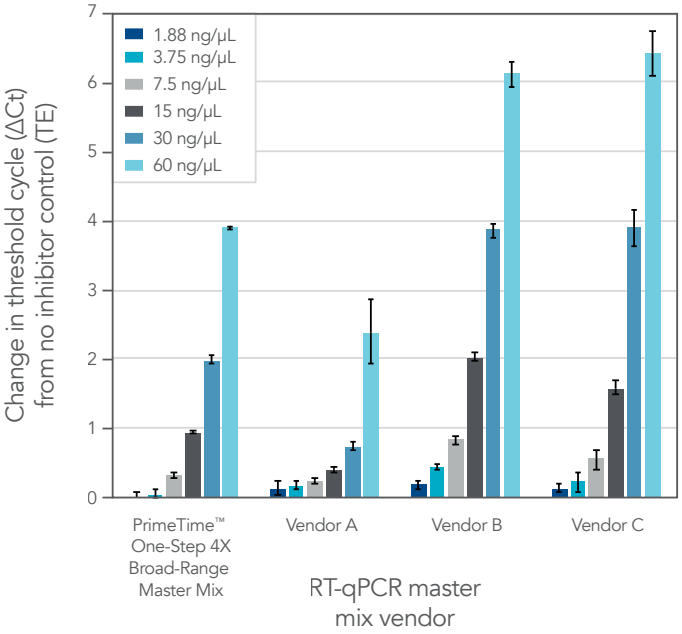

Figure 6. PrimeTime One-Step 4X Broad-Range Master Mix has a higher tolerance to humic acid inhibition than two out of three other commercially available master mixes. RT-qPCR probe assays containing probes and primers specific for Flu A (shown), Flu B, SARS-CoV-2, and RNase P genes were used to amplify RNA template in the presence of humic acid concentrations ranging from 1.88–60 ng/µL. Each master mix was used according to the manufacturer's instructions. RT-qPCR reactions were run on the QuantStudio 7 Flex (n = 6, per inhibitor concentration per master mix). Error bars represent standard deviation.

ORDERING INFORMATION

Product	Quantity*	Catalog number
PrimeTime One-Step 4X Broad-Range Master Mix	1 x 1 mL	10011744
	5 x 1 mL	10011745
	2 x 5 mL	10011746
	5 x 5 mL	10011747
	25 x 10 mL	10011748
	20 x 25 mL	Inquire

* Separate tubes of reference dye and direct amplification enhancer are included with all orders except for the 25 x10 mL and 20 x 25 mL sizes. (Either of these products can be ordered by contacting www.idtdna.com/ContactUs.)

> FOR MORE INFORMATION, VISIT **WWW.IDTDNA.COM/BROADRANGE**.