PrimeTime™ ONE-STEP RT-qPCR MASTER MIX

For probe-based qPCR assays

Achieve high efficiency qPCR with fast or standard cycling protocols

Obtain excellent results in single-plex or multiplex reactions

Efficient single-step workflow for gene expression analysis

EFFICIENT, ONE-STEP, RT-qPCR MASTER MIX

IDT PrimeTime RT-qPCR Master Mix is specifically formulated for one-step RT-qPCR reactions. The 2X mixture of reverse transcriptase, antibody-mediated, hot-start DNA polymerase, dNTPs, buffers, and stabilizers provides reliable performance for analysis of RNA.

LOW INPUT LIMITS

PrimeTime One-Step RT-qPCR Master Mix performance in quantifying an mRNA target, SFRS9, was assessed by RT-qPCR. First, a standard curve was established by making 10-fold serial dilutions of RNA Ultramers™ to SFRS9 (from 10^{10} to 10 copies). Each dilution was mixed with SFRS9-specific primers and probe labeled with 5′ SUN™ fluorophore and a ZEN™/Iowa Black™ FQ double quencher, followed by IDT PrimeTime One-Step Master Mix. As seen in Figure 1, the standard curve (gray line) had an $R^2 = 1$, which demonstrates the ability to amplify low copy samples down to 10 copies of spiked in Ultramers.

The same primer, probe, and master mix were then used to determine the number of copies of SFRS9 mRNA in total human RNA samples from HEK293 cells. PrimeTime One-Step RT-qPCR Master Mix also demonstrates the ability to identify low copy targets in complex samples. For example, it identified SFRS9 RNA in as little as 2 pg of total human RNA (Figure 1). PrimeTime One-Step RT-qPCR Master Mix also demonstrates the ability to identify low copy targets in complex samples. For example, it identified SFRS9 RNA in as little as 2 pg of total human RNA (Figure 1).

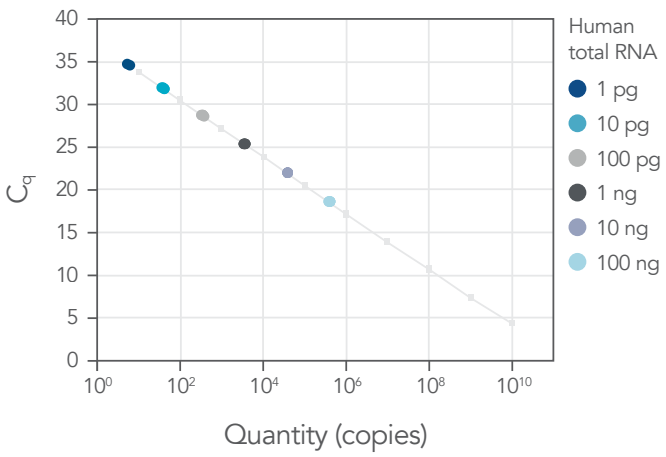

Figure 1. PrimeTime One-Step RT-qPCR Master Mix identify SFRS9 RNA in as little as 2 pg of total human RNA. A PrimeTime qPCR assay containing probes and primers specific for SFRS9 was used to amplify a dilution series of SFRS9 RNA Ultramer™ template to create a standard curve (10^{10} to 10 copies) (gray line), and then a 10-fold dilution series of total RNA from HEK293 cells (100 ng to 1 pg) (rainbow dots).

EXCELLENT PERFORMANCE IN MULTIPLEX REACTIONS

PrimeTime™ One-Step RT-qPCR Master Mix is optimized for gene expression analysis using reverse transcription quantitative PCR (RT-qPCR) and provides high performance in single-plex or multiplex applications. 4 different targets, HPRT, CSK, SFRS9, and CHEK1 were multiplexed with specific primers and probes for each target. Each probe was labeled with distinct fluorophores to avoid overlapping emission spectra.

To establish a standard curve, the multiplexed primer and probes were mixed with serial dilutions of 4 RNA Ultramers designed for each target. The dilutions ranged from 10^{10} copies to 10 copies. The 4-plex results shown in Figure 3 clearly demonstrate that IDT PrimeTime One-Step RT-qPCR Master Mix can amplify each of the 4 targets for each dilution, even the lowest dilution, 10 copies.

The same multiplex assay was used to identify the quantity of each target in 1 ng of HEK293 total RNA, which is plotted on the standard curve with an orange dot (bottom row). As seen above, IDT PrimeTime One-Step Master Mix has a low input limit of 1 ng for all 4 targets, reinforcing that this master mix performance is consistent and maintains the same performance even in highly multiplexed assays (Figure 2).

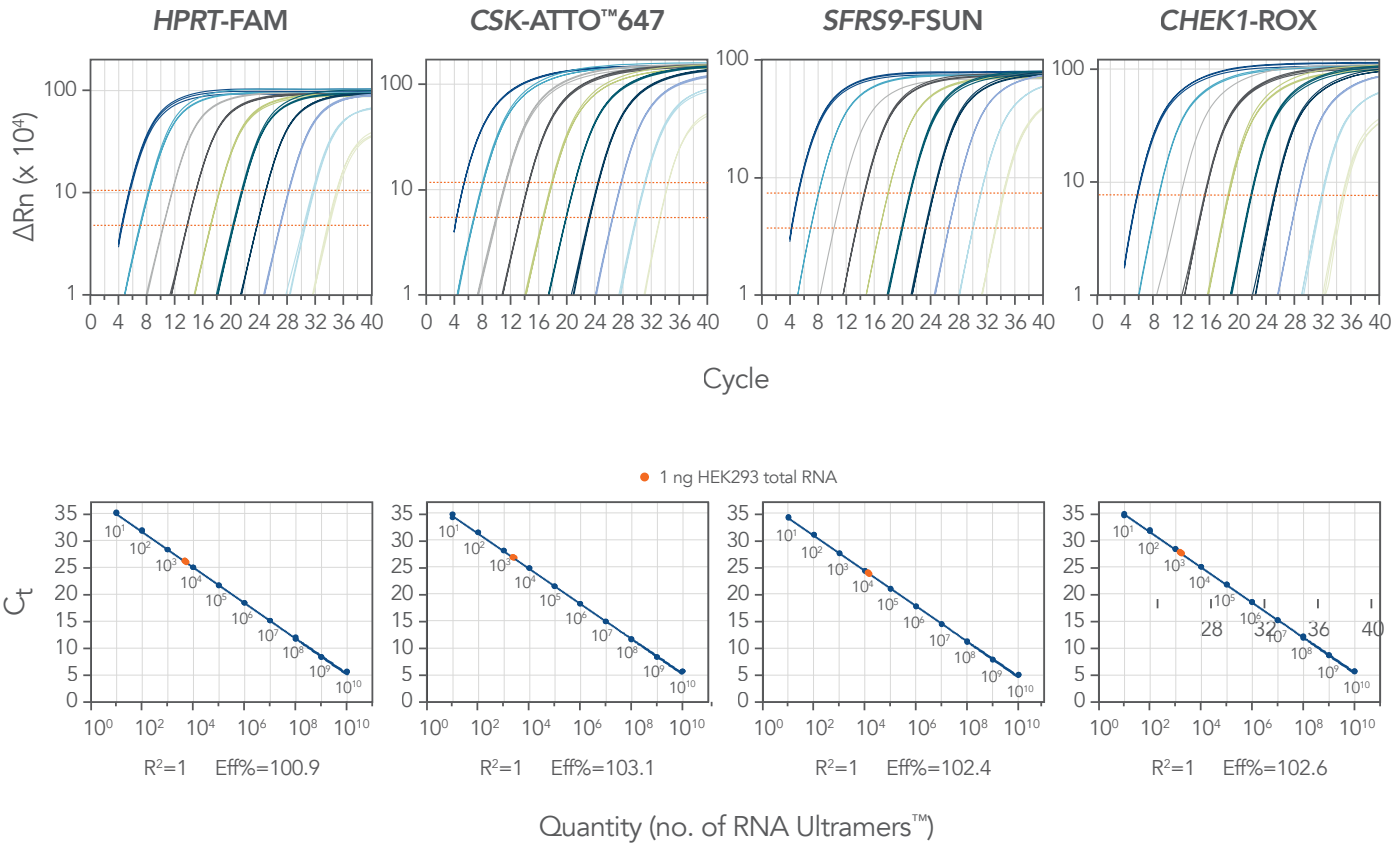

Figure 2. PrimeTime One-Step RT-qPCR Master Mix provides highly sensitive performance in multiplex assays. Multiplex qPCR assays containing probes and primers specific for HPRT-FAM, CSK-ATTO™ 647 (ATTO-TEC GmbH), SFRS9-SUN, and CHEK1-ROX amplified a dilution series of pooled RNA Ultramer templates to create standard curves for each gene. The amount of each transcript in 1 ng of HEK293 total RNA (•) was determined. For each gene, an $R^2 = 1$ and efficiency between 100.9– 103.1% was obtained. Assays were performed on a QuantStudio™ 7 Flex qPCR instrument.

PrimeTime One-Step RT-qPCR Master Mix performs equally well in fast cycling conditions with no loss of efficiency even in this multiplex assay, which is very important for high-throughput settings (Figure 3).

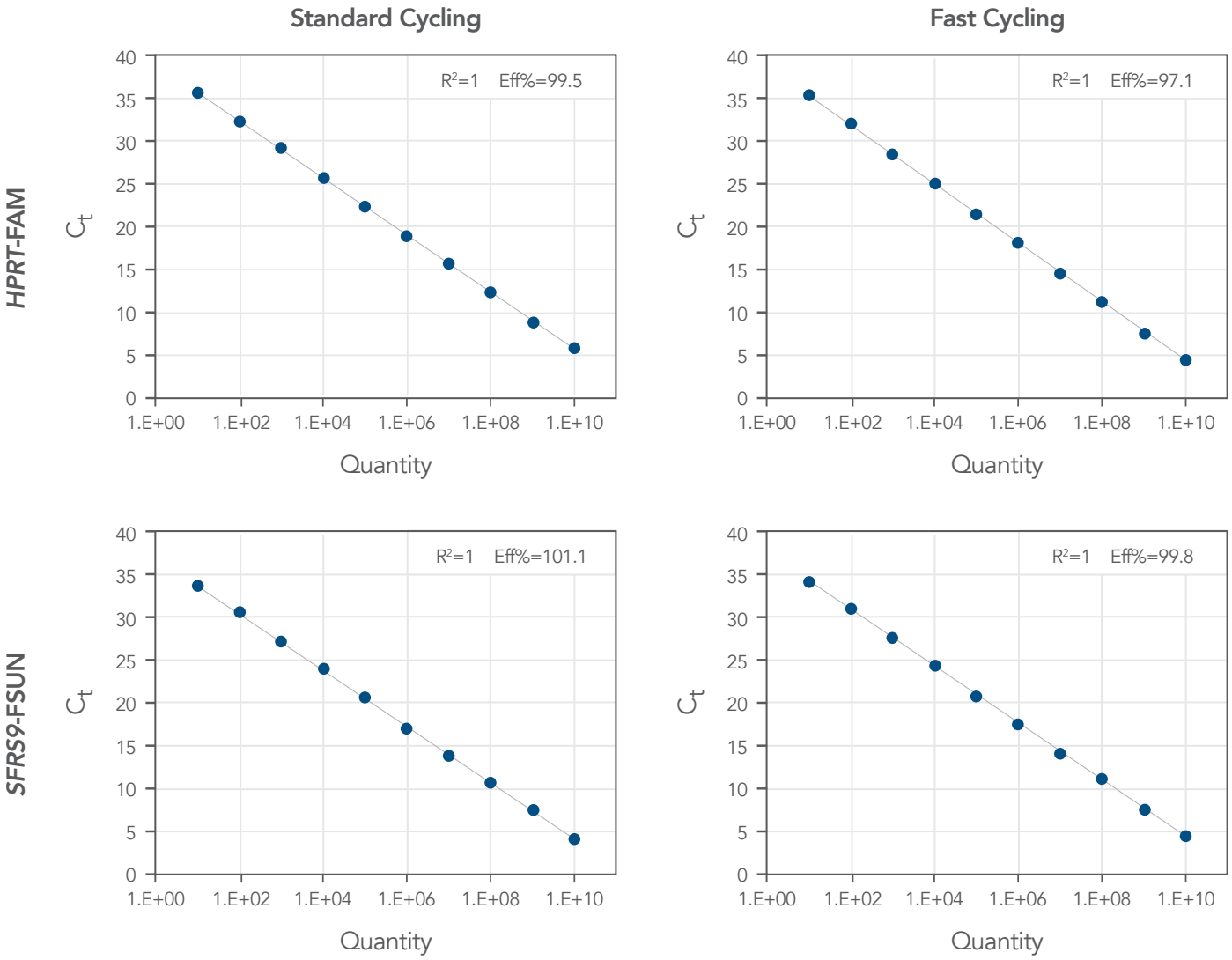

Figure 3. PrimeTime One-Step RT-qPCR Master Mix has no loss of efficiency and detection capabilities in fast qPCR cycling parameters. Multiplexed qPCR assays containing probes and primers specific for *HPRT*-FAM™ and *SFRS9*-SUN™ were used to amplify a dilution series of pooled *HPRT* and *SFRS9* RNA Ultramer™ templates to create a standard curve (10^{10} to 10 copies) using either standard [15 min. 50°C; 3 min. 95°C; 40 x (15 sec. 95°C, 1 min. 60°C] or fast [15 min. 50°C; 3 min. 95°C; 40 x (5 sec. 95°C, 30 sec. 60°C] qPCR cycling parameters. For each standard curve the R^2 = 1 and the efficiency was between 97.1–101.1% demonstrating that there was no loss of performance in fast cycling conditions.

ORDERING INFORMATION

Name	Quantity	Catalog number
PrimeTime™ One-Step RT-qPCR Master Mix*	1x1 mL	10007065
	1x5 mL	10007066
	5x5 mL	10007067
	20x25 mL	10007068
Nuclease-Free Water	10x2 mL	11-04-02-01
	300 mL	11-05-01-14

> FOR MORE INFORMATION GO TO WWW.IDTDNA.COM/ONESTEP