The thermal stability of PrimeTime™ Gene Expression Master Mix enables shipping at ambient temperature, saving laboratory and environmental resources

Abstract

PrimeTime Gene Expression Master Mix has been formulated for probe-based assays in two-step, reverse transcription qPCR (RT-qPCR). This master mix delivers high efficiency qPCR under fast or standard cycling conditions. Here, we show evidence of benchtop stability and consistent qPCR results in single-plex and duplex reactions after the master mix has been exposed to extreme temperature conditions (50°C for up to 7 days; up to 20 freeze-thaw cycles). These conditions exceed those experienced by the master mix during routine shipping or laboratory use. These results show that our master mix can be shipped at ambient temperatures, providing environmental benefits and research dollar savings.

Introduction

Since its beginnings, IDT has striven to improve sustainability practices, and our manufacturing facilities (Coralville, IA and San Diego, CA) have obtained ISO 14001:2004 certification for environmental management systems. Much of the focus has been on manufacturing processes to reduce air emissions, water and energy usage, and hazardous and landfill waste production. Here, we show results from experiments supporting ambient temperature shipping for PrimeTime Gene Expression Master Mix to further mitigate environmental impacts of our products.

Eliminating shipping in insulated containers with gel packs or dry ice reduces shipping costs by 35–70% (depending on your location) and may shorten delivery times. Ambient shipping also lessens detrimental environmental impacts in several ways, such as:

- Eliminating manufacturing resources required for insulated containers, dry ice, and gel packs
- Eliminating waste disposal of insulated containers or gel packs in landfills or incinerators
- Decreasing transportation resources (e.g., fuel consumption and greenhouse gas emissions) by decreasing shipping weight

For consistency and convenience, many researchers use commercial master mixes that have been designed with enhancers and stabilizers. IDT scientists have developed a thermostable qPCR master mix for probe-based assays. The versatile PrimeTime Gene Expression Master Mix (Cat # 1055770, 1055772, or 1055771) produces consistent results under both standard and fast cycling conditions and is compatible with a wide range of real-time PCR platforms.

We show that PrimeTime Gene Expression Master Mix is also compatible with overnight and high throughput experiments, based on the benchtop stability of the master mix. We have further extended our thermal stability testing to show that function of this master mix is not negatively affected by conditions that could be encountered during ambient shipping.

Results and discussion

High PCR efficiency after extended exposure to benchtop temperatures

We have shown that 3 batches of PrimeTime Gene Expression Master Mix produce reliable results, whether the qPCR was run immediately after setup or after the reactions were maintained at room temperature for 24 hr (Figure 1).

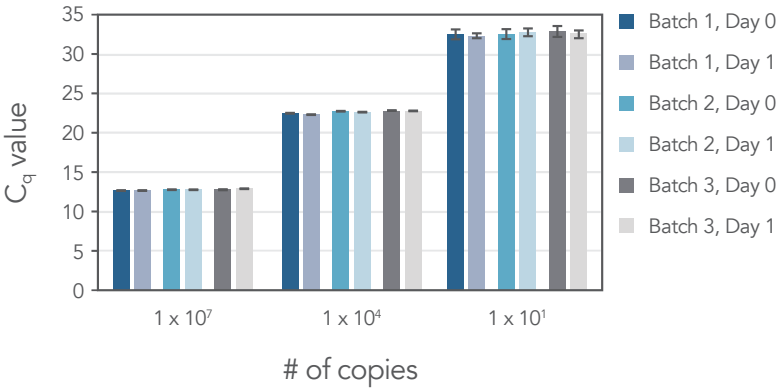
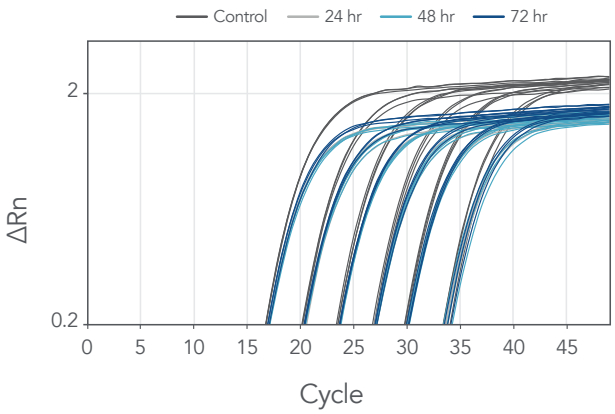


Figure 1. Consistent and precise results after 24 hr at room temperature using different batches of PrimeTime Gene Expression Master Mix. qPCRs consisting of the PrimeTime qPCR Assay targeting *HPRT*, PrimeTime Gene Expression Master Mix, reference dye, and varying amounts of gBlocks™ Gene Fragments (3 of 7 dilutions shown: 10^7–10^1 copies, 8 replicates) were run immediately (Day 0) or after 24 hr (Day 1). The ΔC_q values for template levels >10 copies were <0.5. To see additional results, visit www.idtdna.com/qPCRmastermix.

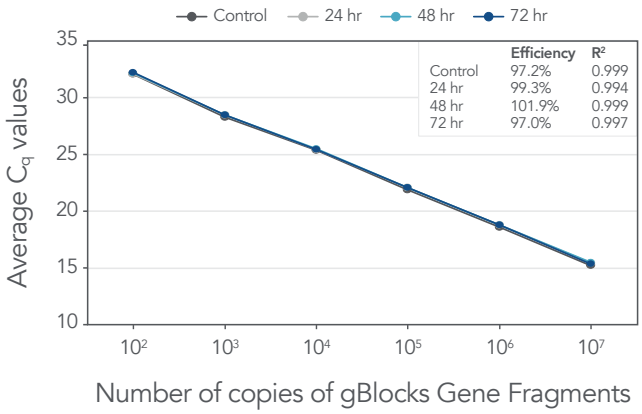
We also observed similar, consistent results after testing 3 additional batches of master mix with other qPCR reactions targeting *TFRC, ACN9, B2M, IPO8, POLR2A* and *HMBS*, run as single-plex and duplex reactions after extended benchtop time up to 72 hr (Figure 2). For all 6 assays, qPCR efficiency was in the range of 84.9–103.4% (R^2 >0.991) under single-plex and duplex conditions. When comparing the controls (qPCRs run immediately after reaction setup) to reactions held for 24, 48, or 72 hr at room temperature before cycling, ΔC_q values were <0.5, with a few exceptions (see Figure 2C for representative data). Together, these results indicate that there are no identifiable detrimental effects from maintaining the qPCR at room temperature for up to 72 hr.

Our results show consistent qPCR results even after the reaction remained at room temperature for up to 72 hr. This highlights the stability of PrimeTime Gene Expression Master Mix and supports the use of this master mix in overnight or automated, high throughput experiments.

A. Amplification plot (PrimeTime *ACN9* qPCR Assay)



B. Standard curve (PrimeTime *ACN9* qPCR Assay)



C. ΔC_q = Average C_q (control) – Average C_q (72 hr)

	ΔC_q, PrimeTime qPCR Assays							
# of copies of gBlocks Gene Fragments	*HPRT*	*HPRT* (duplexed with *GUSB*)	*GUSB*	*GUSB* (duplexed with *HPRT*)	*TFRC*	*TFRC* (duplexed with *ACN9*)	*ACN9*	*ACN9* (duplexed with *TFRC*)
10^7	–0.08	–0.21	**–0.55**	–0.22	–0.19	–0.13	–0.15	–0.23
10^6	0.04	–0.20	–0.03	–0.19	–0.07	–0.06	–0.15	–0.16
10^5	–0.34	–0.27	–0.22	–0.16	–0.01	–0.05	–0.15	–0.24
10^4	–0.10	–0.29	–0.45	–0.27	0.07	–0.02	–0.04	–0.16
10^3	–0.05	–0.38	–0.16	–0.25	–0.08	–0.24	–0.19	–0.29
10^2	–0.22	0.04	–0.09	–0.35	–0.40	–0.13	–0.17	0.14
Average | Δ C_q |	**0.14**	**0.23**	**0.25**	**0.24**	**0.14**	**0.10**	**0.14**	**0.20**
# of copies of gBlocks Gene Fragments	*B2M*	*B2M* (duplexed with *IPO8*)	*IPO8*	*IPO8* (duplexed with *B2M*)	*POLR2A*	*POLR2A* duplexed with *HMBS*)	*HMBS*	*HMBS* (duplexed with *POLR2A*)
10^7	0.01	–0.08	–0.10	–0.12	–0.07	–0.11	**0.62**	0.30
10^6	–0.01	–0.08	–0.14	–0.10	–0.06	0.04	0.07	0.15
10^5	–0.04	–0.06	–0.09	–0.08	–0.01	0.07	–0.01	0.14
10^4	–0.17	–0.09	–0.16	–0.13	0.01	0.05	0.15	0.22
10^3	–0.01	0.03	–0.21	–0.08	–0.15	0.06	0.12	0.28
10^2	0.15	–0.01	**–0.62**	–0.28	–0.13	0.03	0.01	0.34
Average | Δ C_q |	**0.07**	**0.06**	**0.22**	**0.13**	**0.07**	**0.06**	**0.16**	**0.24**

Figure 2. PrimeTime Gene Expression Master Mix has excellent benchtop stability—consistent, high PCR efficiency was observed after reactions were set up and run after 24, 48, and 72 hr at room temperature. qPCRs included PrimeTime Gene Expression Master Mix and PrimeTime qPCR Assays (*ACN9, TFRC, HPRT, GUSB, B2M, IPO8, POLR2A*, and *HMBS*) in single-plex and duplex. The qPCRs were set up and either run immediately (control) or remained at room temperature for 24, 48, or 72 hr before cycling. Consistent, high efficiency PCR results are shown by representative amplification curves (**A**) and standard curves (**B**) from the qPCR reaction targeting *ACN9* run in single-plex: controls (dark gray) and 24, 48, and 72 hr samples (light gray, light blue, and dark blue, respectively). (**C**) Representative data comparing ΔC_q values of the control qPCRs to reactions held for 72 hr at room temperature show that the difference in the average ΔC_q values from technical triplicates were within ±0.50, except in 3 instances (dark blue).

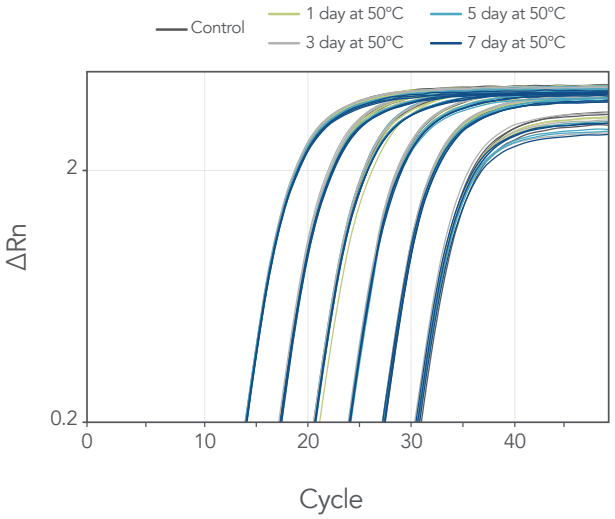
Consistent C_q values after extended heat-stress at 50°C

To further characterize the dependability and consistency of PrimeTime Gene Expression Master Mix, we examined data from accelerated stability experiments. The extreme conditions, which would not be encountered during normal product life and use, included elevated temperatures for extended periods of time.

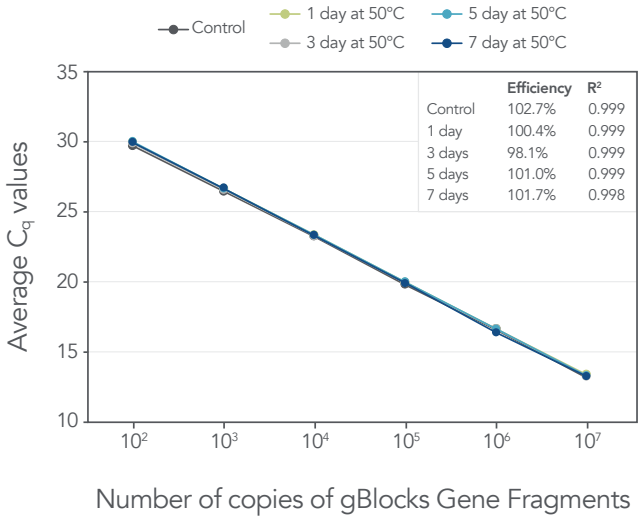
Three batches of master mix were heated at 50°C for 1, 3, or 7 days before use in qPCR experiments (**Figure 3**). We chose *HPRT* and *GUSB* as our representative qPCR targets for this analysis. High PCR efficiency was observed (range of 95.1–103.6% with R^2 >0.991) with all targets tested. When comparing the controls (containing frozen, unheated master mix) to reactions containing heat-treated master mix, ΔC_q values were <0.5, with a few exceptions that were primarily seen when using a low amount of starting template (see **Figure 3C** for representative data). The small ΔC_q values emphasize the durability of the PrimeTime Gene Expression Master Mix after extreme thermal stress.

Our results consistently show successful qPCR results even after PrimeTime Gene Expression Master Mix was heated at 50°C for 7 days, which emphasizes the stability of the master mix and its components when not stored at –20°C.

A. Amplification plot (PrimeTime *HPRT* qPCR Assay)



B. Standard curve (PrimeTime *HPRT* qPCR Assay)



C. ΔC_q = Average C_q (controls) – Average C_q (X days)

	ΔC_q, *HPRT*				ΔC_q, *HPRT* (duplexed with *GUSB*)			
# of copies of gBlocks Gene Fragments	Control vs. 1 day at 50° C	Control vs. 3 days at 50° C	Control vs. 5 days at 50° C	Control vs. 7 days at 50° C	Control vs. 1 day at 50° C	Control vs. 3 days at 50° C	Control vs. 5 days at 50° C	Control vs. 7 days at 50° C
10^7	–0.06	–0.15	–0.02	–0.01	–0.11	–0.09	0.06	0.03
10^6	–0.07	–0.14	–0.06	0.03	–0.10	–0.13	–0.04	0.12
10^5	–0.05	–0.16	–0.02	0.05	–0.09	–0.16	0.03	0.05
10^4	–0.08	–0.14	–0.03	0.06	–0.06	–0.02	0.14	0.11
10^3	0.05	–0.12	–0.01	0.03	–0.01	0.00	0.01	0.14
10^2	–0.07	–0.08	–0.04	–0.03	–0.11	–0.02	0.00	0.00
Average | Δ C_q |	**0.06**	**0.13**	**0.03**	**0.04**	**0.08**	**0.07**	**0.05**	**0.08**
	ΔC_q, *GUSB*				ΔC_q, *GUSB* (duplexed with *HPRT*)			
# of copies of gBlocks Gene Fragments	Control vs. 1 day at 50° C	Control vs. 3 days at 50° C	Control vs. 5 days at 50° C	Control vs. 7 days at 50° C	Control vs. 1 day at 50° C	Control vs. 3 days at 50° C	Control vs. 5 days at 50° C	Control vs. 7 days at 50° C
10^7	–0.06	–0.04	0.04	0.10	–0.06	–0.06	0.13	0.01
10^6	0.20	0.19	0.37	0.35	0.08	0.03	0.21	0.27
10^5	–0.03	0.03	0.12	0.09	–0.02	–0.03	0.15	0.08
10^4	–0.02	–0.02	0.18	0.14	0.05	0.05	0.22	0.20
10^3	0.13	0.18	0.27	0.25	0.04	–0.13	0.13	0.03
10^2	0.10	0.28	0.08	0.29	0.18	0.13	0.22	0.06
Average | Δ C_q |	**0.09**	**0.12**	**0.18**	**0.20**	**0.07**	**0.07**	**0.18**	**0.11**

Figure 3. Even after exposure to elevated temperatures (50°C for up to 7 days), the PrimeTime Gene Expression Master Mix provides high PCR efficiency with no change in C_q values, when compared to untreated master mix. PrimeTime Gene Expression Master Mix was incubated at 50°C for 1, 3, 5, or 7 days (experimental), or stored at –20°C until use (control). qPCRs included the heat-treated or control master mix and PrimeTime qPCR Assays (*HPRT* and *GUSB*) in single-plex and duplex. Consistent, high efficiency PCR results are shown by representative amplification curves (**A**) and standard curves (**B**) from *HPRT* (single-plex) that remained at room temperature for 24 hr before running the thermal cycler: control master mix (dark gray) and heat-exposed master mix (1 day, green; 3 days, light gray; 5 days, light blue; and 7 days, dark blue). (**C**) Representative data comparing ΔC_q values of the control qPCRs to reactions that included 1-, 3-, 5-, or 7-day, heat-exposed master mix show that the difference in the average C_q values from technical triplicates were within ±0.50.

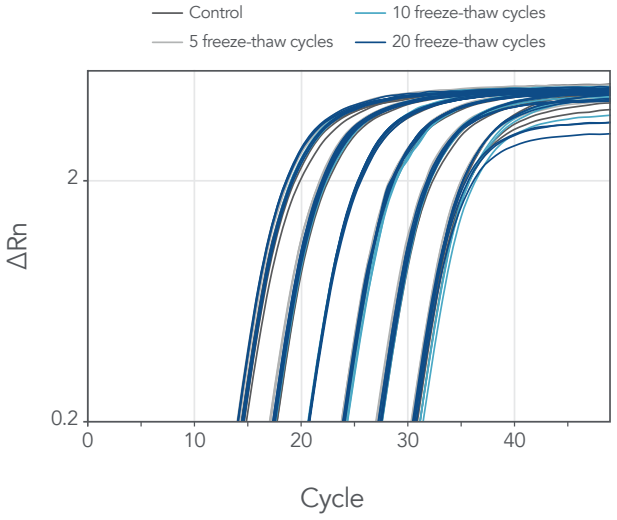
Consistent C_q values after multiple freeze-thaw cycles

We also tested the effect of repeated freezing and thawing on the PrimeTime Gene Expression Master Mix. Freeze-thawing can occur during normal use in the laboratory or, potentially, during shipping. We tested up to 20 freeze-thaw cycles, which is more extreme than typical laboratory usage.

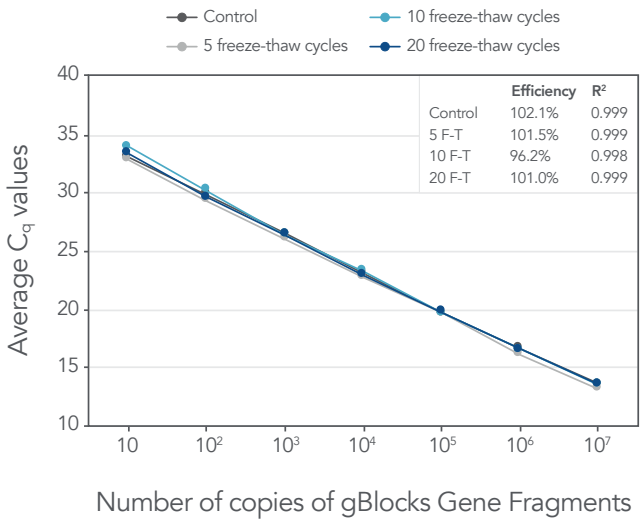
PrimeTime Gene Expression Master Mix was frozen and thawed 5, 10, or 20 times before use in qPCR experiments (Figure 4). We chose *HPRT* and *GUSB* as our representative qPCR targets for this analysis. High PCR efficiency was observed (range of 95.4–104.9% with R^2 >0.994) with all qPCRs tested. When comparing the control qPCRs (containing frozen master mix) to reactions containing frozen-thawed master mix, ΔC_q values were all <0.5, with a few exceptions (see Figure 4C for representative data). The small ΔC_q values indicate essentially no detrimental effects from repeated freeze-thawing of PrimeTime Gene Expression Master Mix.

These results show that efficiency of the qPCR was unaffected, even after the PrimeTime Gene Expression Master Mix was subjected to as many as 20 freeze-thaw cycles, which further emphasizes the stability of the master mix and its components.

A. Amplification plot (PrimeTime *HPRT* qPCR Assay)



B. Standard curve (PrimeTime *HPRT* qPCR Assay)



C. ΔC_q = Average C_q (controls) – Average C_q (X freeze-thaw cycles)

	HPRT			*HPRT* (duplexed with *GUSB*)		
# of copies of gBlocks Gene Fragments	Control vs. 5 freeze-thaw cycles	Control vs. 10 freeze-thaw cycles	Control vs. 20 freeze-thaw cycles	Control vs. 5 freeze-thaw cycles	Control vs. 10 freeze-thaw cycles	Control vs. 20 freeze-thaw cycles
10^7	0.49	0.13	0.08	–0.01	–0.04	0.04
10^6	**0.56**	0.13	0.15	0.12	–0.10	–0.13
10^5	0.01	0.03	–0.06	–0.34	–0.10	–0.13
10^4	0.32	–0.18	0.14	0.21	–0.19	–0.09
10^3	0.47	0.07	0.09	0.44	–0.21	0.28
10^2	**0.53**	–0.33	0.32	–0.12	–0.22	–0.26
Average | Δ C_q |	**0.40**	**0.14**	**0.14**	**0.21**	**0.14**	**0.15**
	GUSB			*GUSB* (duplexed with *HPRT*)		
# of copies of gBlocks Gene Fragments	Control vs. 5 freeze-thaw cycles	Control vs. 10 freeze-thaw cycles	Control vs. 20 freeze-thaw cycles	Control vs. 5 freeze-thaw cycles	Control vs. 10 freeze-thaw cycles	Control vs. 20 freeze-thaw cycles
10^7	0.12	–0.08	0.09	0.02	–0.07	0.05
10^6	0.13	0.13	0.14	0.07	–0.05	–0.04
10^5	0.04	0.12	0.08	–0.31	–0.06	–0.02
10^4	0.46	0.02	0.23	0.22	–0.19	–0.02
10^3	0.16	0.11	0.27	0.44	–0.19	0.31
10^2	0.19	0.05	0.00	0.18	–0.06	0.02
Average | Δ C_q |	**0.18**	**0.09**	**0.14**	**0.21**	**0.11**	**0.08**

Figure 4. Multiple freeze-thaw cycles (up to 20) do not affect the functionality of the PrimeTime Gene Expression Master Mix, which still results in consistent C_q values and high PCR efficiency. PrimeTime Gene Expression Master Mix underwent 5, 10, or 20 freeze-thaw cycles, while control master mix was stored frozen (–20°C) until use. qPCRs included the freeze-thawed or control master mix and PrimeTime qPCR Assays (*HPRT* and *GUSB*) in single-plex and duplex. Consistent, high efficiency PCR results are shown by representative amplification curves (**A**) and standard curves (**B**) from *HPRT* targeting reactions (single-plex) that remained at room temperature for 24 hr before running the thermal cycler: control master mix (dark gray) and the treated master mix (5 freeze-thaw cycles, light gray; 10 cycles, light blue; 20 cycles, dark blue). (**C**) Representative data comparing ΔC_q values of the control qPCRs to reactions that included frozen and thawed master mix show that the difference in the average C_q values from technical triplicates were within ±0.50, except in 2 instances (dark blue). F-T = freeze-thaw.

Case study

Thus far, the results from our experiments indicate that all the components of the master mix, including the hot-start polymerase, are stable under varying temperature conditions. We have tested 2 batches of PrimeTime Gene Expression Master Mix that were either stored at –20°C (control) or shipped at ambient temperatures. Roundtrip shipments from IDT facilities in the USA to Singapore or Australia took 7 or 8 days, respectively. Data about ambient conditions were available for the first 2 days of transit to Singapore. These records indicated that the temperature varied between 14 and 24°C and pressure varied between 800 and 1050 mBar.

We chose *HPRT* and *GUSB* as representative qPCR targets to compare control master mix (held at a constant –20°C) and experimental master mix (shipped at ambient temperature). High PCR efficiency was observed (range of 97– 101%, R^2 >0.99) with all targets tested. When comparing the control reactions containing frozen, unshipped master mix to reactions containing shipped master mix, ΔC_q values were all <0.50. The small ΔC_q values indicate that the master mix was not compromised by ambient shipping.

Environmental benefits of shipping at ambient temperature vs. using dry ice

To illustrate the environmental impact of switching to ambient shipping, consider that each package shipped with dry ice carries an extra 1.5–6 kg (3–13 lbs) of gross weight. In a typical year for IDT, this can mean a decrease of ~30,400 kg (~67,000 lbs) in total package weight if 10,000 orders were shipped at ambient temperature instead of on dry ice. Also, assuming cardboard packaging is recycled, ambient shipping materials would not create landfill waste, while dry-ice shipping materials include 28.5 tons of dry ice and would create ~437 m^2 (~4700 ft^2) of landfill waste from the expanded polystyrene (foam) containers.

Conclusions

PrimeTime Gene Expression Master Mix has proven to be functional under various temperature conditions, which supports shipping at ambient temperature, as well as use in overnight or high throughput experiments. Consistently high PCR efficiencies and reproducible C_q results are observed even under the following conditions:

- The qPCR reactions, including the master mix, were set up and remained at room temperatures for up to 72 hr.
- The Master Mix was stored at elevated temperatures for extended periods of time (up to 7 days at 50°C).
- The Master Mix was subjected to 20 freeze-thaw cycles.

Using the versatile PrimeTime Gene Expression Master Mix in your gene expression studies lets you focus on your results instead of troubleshooting your qPCR conditions. Ambient shipping saves packaging and transportation resources, and greatly reduces solid waste. Because our experiments show that PrimeTime Gene Expression Master Mix is not adversely affected by shipping at ambient conditions, we have moved to shipping at ambient temperature, which is better for your budget and the environment.

Visit **www.idtdna.com/qPCRMasterMix** for more information, or **contact us** with questions.

Methods

Heat stressing the master mix

Vials of PrimeTime Gene Expression Master Mix from 3 batches were heated in a 50°C incubator for 1, 3, and 7 days. One of the 3 batches was incubated at 50°C for 14 days with similar results (data not shown). The unheated control master mix was stored at –20°C.

Freeze-thawing the master mix

PrimeTime Gene Expression Master Mix (1.5 mL vials) were subjected to 5, 10, and 20 freeze-thaw cycles. Each cycle consisted of thawing the master mix at room temperature for at least 1 hr, followed by freezing at –20°C for at least 12 hr. The unheated control master mix was stored at –20°C.

qPCR

All experiments shown were run on an Applied Biosystems® 7900HT Real-Time PCR System (Thermo Fisher Scientific) under fast cycling conditions (Table 1). Eight replicate qPCRs run as single-plex or duplexed reactions were used in the experiments for Figure 1, while triplicate qPCRs were run as single-plex or duplexed reactions for the remaining experiments. Details of the qPCRs are provided in Table 1 and 2. For some experiments, the single-plex or duplex PCR mix was left on the benchtop (room temperature, 15–25°C) for 24, 48, or 72 hr before running on the thermal cycler (Figure 2). For other experiments, the master mix was subjected to temperature stress (50°C heating or multiple freeze-thaw cycles) before use, where the qPCR reactions were run immediately after set up (data not shown) or after 24 hr at room temperature (Figures 3 and 4).

gBlocks Gene Fragments, which are sequence verified, double-stranded DNA, were used as the qPCR template. Ten-fold dilutions were made from 10^7 to 10 copies per reaction. Some experiments were also performed with cDNA prepared from commercially available universal RNA using the AffinityScript® RT-PCR Kit (Agilent Technologies). The results with cDNA template (5-fold dilutions from 50 to 0.0032 ng) were similar to those with gBlocks Gene Fragments templates (data not shown).

ΔC_q was calculated from the average C_qs of replicate reactions for the conditions being studied (e.g., qPCR results from different time points or from different temperature storage conditions).

Table 1. qPCR setup.

	Single-plex	Single-plex	Duplex
Reaction 1—predesigned PrimeTime primers and FAM-labeled probe (1X final)	0.3 µL	—	0.3 µL
Reaction 2—predesigned PrimeTime primers and HEX-labeled probe (1X final)	—	0.3 µL	0.3 µL
PrimeTime Gene Expression Master Mix* (1X final)	6 µL	6 µL	6 µL
Reference dye (0.5 µM final)	0.24 µL	0.24 µL	0.24 µL
Nuclease-Free Water	0.46 µL	0.46 µL	0.16 µL
Template (gBlocks Gene Fragments) (10^1–10^7 copies)	5 µL	5 µL	5 µL
Reaction volume	**12 µL**	**12 µL**	**12 µL**

* PrimeTime Gene Expression Master Mix includes a separate tube of reference dye for use as needed. IDT offers Nuclease-Free Water (Cat # 11-04-02-01). Fast cycling conditions: 3 min. 95°C; 49 x (5 sec. 95°C, 30 sec. 60°C).

Table 2. qPCR targets and template details.

Component	Detailed information		
Primers and probes	PrimeTime qPCR Assays: visit www.idtdna.com/PrimeTime for more information. The following pairs of predesigned sequences were run in single-plex or duplex:		
	Target	**Probe fluorophore and quenchers**	**Sequence ID**
	HPRT	FAM/ZEN/IBFQ	Hs.PT.58v.45621572
	GUSB	HEX/ZEN/IBFQ	Hs.PT.58v.27737538
	TFRC	FAM/ZEN/IBFQ	Hs.PT.58.3164874
	ACN9	HEX/ZEN/IBFQ	Hs.PT.58.588601
	B2M	FAM/ZEN/IBFQ	Hs.PT.58v.18759587
	IPO8	HEX/ZEN/IBFQ	Hs.PT.58.3371286
	POLR2A	FAM/ZEN/IBFQ	Hs.PT.58.25515089
	HMBS	HEX/ZEN/IBFQ	Hs.PT.58.40437381
Templates	gBlocks Gene Fragments: visit www.idtdna.com/gBlocks for more information.		
	***HPRT*, 200 bp (5′→3′):**		
	TGTTGGATTT GAAATTCCAG ACAAGTTTGT TGTAGGATAT GCCCTTGACT ATAATGAATA CTTCAGGGAT TTGAATCATG TTTGTGTCAT TAGTGAAACT GGAAAAGCAA AATACAAAGC CTAAGATGAG AGTTCAAGTT GAGTTTGGAA ACATCTGGAG TCCTATTGAC ATCGCTTGAC AAATTATCAA TGTTCTAGTT		
	***GUSB*, 170 bp (5′→3′):**		
	ACTGGTATAA GAAGTATCAG AAGCCCATTA TTCAGAGCGA GTATGGAGCA GAAACGATTG CAGGGTTTCA CCAGGATCCA CCTCTGATGT TCACTGAAGA GTACCAGAAA AGTCTGCTAG AGCAGTACCA TCTGGGTCTG GATCAAAAAC GCAGAAAATA CGTGGTTGGA		
	***TFRC*, 160 bp (5′→3′):**		
	GTACAACAGC CAACTGCTTT CATTTGTGAG GGATCTGAAC CAATACAGAG CAGACATAAA GGAAATGGGC CTGAGTTTAC AGTGGCTGTA TTCTGCTCGT GGAGACTTCT TCCGTGCTAC TTCCAGACTA ACAACAGATT TCGGGAATGC TGAGAAAACA		
	***ACN9*, 160 bp (5′→3′):**		
	GGGCGACCAG TACGTGAAAG ACGAATTTAG GAGACATAAG ACCGTTGGTT CTGACGAGGC ACAGCGTTTC TTGCAAGAAT GGGAGGTGTA TGCAACAGCG TTATTGCAAC AGGCTAACGA AAACAGACAA AATTCAACTG GAAAAGCATG TTTTGGCACC		
	***B2M*, 170 bp (5′→3′):**		
	TGTCTTTCAG CAAGGACTGG TCTTTCTATC TCTTGTACTA CACTGAATTC ACCCCCACTG AAAAAGATGA GTATGCCTGC CGTGTGAACC ATGTGACTTT GTCACAGCCC AAGATAGTTA AGTGGGATCG AGACATGTAA GCAGCATCAT GGAGGTTTGA AGATGCCGCA		
	***IPO8*, 200 bp (5′→3′):**		
	ATGTGGCAGC TTCTAGGTAT ACTATATGAA GTGTTTCAGC AGGATTGCTT TGAATACTTT ACAGACATGA TGCCTCTCCT GCATAATTAT GTGACAATAG ATACAGATAC CTTACTATCA AATGCAAAAC ATTTAGAAAT TCTTTTTACA ATGTGTAGGA AGGTACTATG TGGAGATGCA GGAGAAGATG CAGAGTGTCA		
	***POLR2A*, 160 bp (5′→3′):**		
	GATGACAATG CAGAGAAGCT GGTGCTCCGT ATTCGCATCA TGAACAGCGA TGAGAACAAG ATGCAAGAGG AGGAAGAGGT GGTGGACAAG ATGGATGATG ATGTCTTCCT GCGCTGCATC GAGTCCAACA TGCTGACAGA TATGACCCTG CAGGGCATCG		
	***HMBS*, 180 bp (5′→3′):**		
	ACTCCTTGAA GGACCTGCCC ACTGTGCTTC CTCCTGGCTT CACCATCGGA GCCATCTGCA AGCGGGAAAA CCCTCATGAT GCTGTTGTCT TTCACCCAAA ATTTGTTGGG AAGACCCTAG AAACCCTGCC AGAGAAGAGT GTGGTGGGAA GTGGTGGGAA GCGAAGAGCA GCCCAGCTGC		

Revision history

Version	Date released	Description of changes
3	February 2023	Updated text for regulatory compliance
2	April 2019	Corrected sequence of the *HPRT* template on page 10
1.1	February 2017	Updated with new IDT logo and styles
1	May 2016	Original white paper

The thermal stability of PrimeTime™ Gene Expression Master Mix enables shipping at ambient temperature, saving laboratory and environmental resources

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Doc ID: RUO22-1613_001 03/23

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