PrimeTime® qPCR Primers

For Intercalating Dye Assays

PrimeTime qPCR Primer Attributes

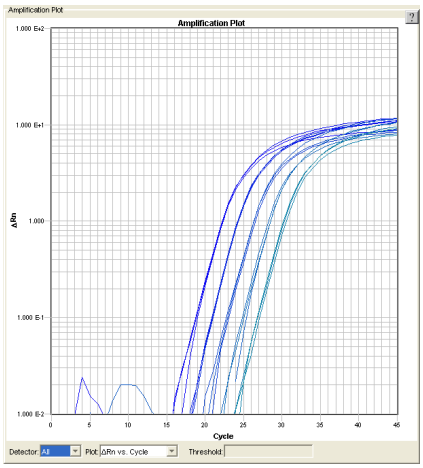
PrimeTime qPCR Primers provide the same primer pairs found in the IDT PrimeTime qPCR Assays designed to detect genes in human, mouse, and rat transcriptomes. These primer sets are ideal for use with SYBR® Green, EvaGreen®, and other intercalating dyes, where no probe is needed.

- ***Up-to-date sequence design***—Primers synthesized at time of order using current sequence information to avoid SNPs and BLAST searched to eliminate off-target effects
- ***Guaranteed performance, high efficiency***—qPCR efficiencies average >90% and PCR products consistently yield single bands upon gel analysis
- ***Compatible with popular master mixes***—Achieve comparable efficiencies when used with various commercial master mixes under manufacturers' cycling conditions
- ***Easy ordering and sequence information***—Simple assay selection interface with sequences provided upon order placement

Easy Transition from Intercalating Dyes to 5' Nuclease Assays

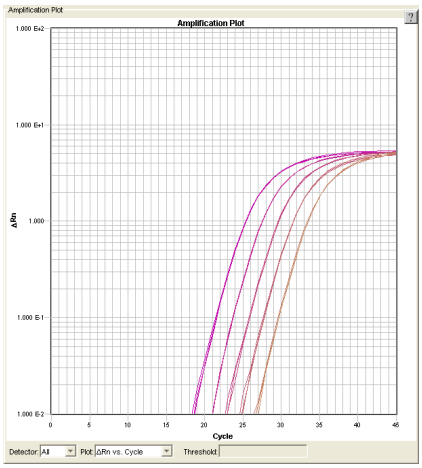
These pre-designed primer sets eliminate the inconvenience of designing specific assays for intercalating dyes. And since both intercalating dye and probe-based pre-designed assays use the same primers, the transition from discovery to screening is simplified. Test hundreds of transcripts with intercalating dye assays and then upgrade to probe-based assays for improved specificity and performance as sample numbers decrease.

The efficiencies for 15 randomly selected qPCR assays with probe (PrimeTime qPCR Assays) and without (PrimeTime qPCR Primers) were compared and demonstrated that the primers work effectively in either format (Figure 1). Efficiencies for all assays, regardless of whether intercalating dye or probe was used, were consistently >90%.



Panel A. Intercalating Dye (SYBR Green).

Figure 1. PrimeTime® qPCR Assays Yield the Same High Efficiency Whether Used With Intercalating Dyes or Probe. Amplification of 5 sequential 4-fold dilutions of cDNA using PrimeTime qPCR Primers (with SYBR Green) or the PrimeTime qPCR Assay (with dual-labeled probe) to human 3-oxo-acid CoA transferase 1 (OXCT1) (NM_000436).



Panel B. Dual-labeled Probe.

PrimeTime® qPCR Primers

Guaranteed Performance

Pre-designed, and synthesized at the time of order, PrimeTime qPCR Primers incorporate up-to-date sequence information and accurate T_m and secondary structure prediction. They are also BLAST searched to protect against off-target amplification and to avoid SNPs. Experimental data generated on a leading qPCR instrument showed that intercalating assays run with PrimeTime qPCR Primers consistently generate qPCR reaction efficiencies >90% (Figure 2) when used with a commercially available master mix, and measured over a minimum of 4 orders of magnitude. PCR products analyzed by gel electrophoresis yield single bands.

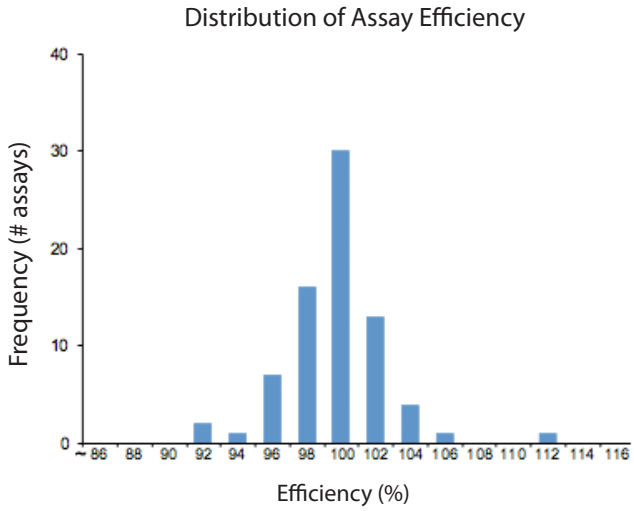


Figure 2. PrimeTime® qPCR Primer Assays Have Average Reaction Efficiency >90%. 60 randomly selected PrimeTime qPCR Primer Assays and 15 PrimeTime qPCR Primer Assays for endogenous control genes used with Brilliant III Ultra Fast SYBR® Green qPCR Master Mix (Agilent) were analyzed over 5 sequential 4-fold dilutions (from 50–0.195 ng/reaction) of cDNA prepared from Universal Human Reference RNA (Agilent). Reactions were run on the 7900HT Fast Real-Time PCR System (Applied Biosystems) using PCR cycling conditions: 3 min 95°C; 45 x (5 sec. 95°C, 15 sec. 60°C). Average reaction efficiencies for the assays tested here exceeded 98%.

Compatible with Popular Master Mixes

PrimeTime qPCR Primers are compatible with leading qPCR master mixes and have been successfully used under both normal and fast cycling parameters. Table 1 shows efficiency data from 15 randomly selected PrimeTime qPCR Primer assays when used with 5 popular commercial master mixes. Again, all assay efficiencies were >90%.

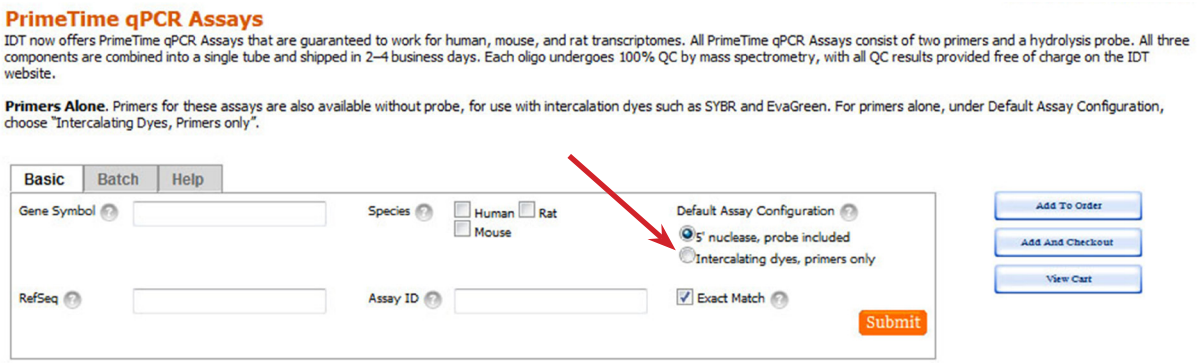
Commercial Master Mix	Mean Efficiency (%)	Successful Reactions (%)
Agilent Brilliant III Ultra-Fast SYBR® Green qPCR	99.1	100
Applied Biosystems POWER SYBR® Green qPCR	98.2	100
Bio-Rad iQ SYBR® Green Supermix	97.4	100
Roche FastStart SYBR® Green	97.9	100
Quanta PerfeCTa SYBR® Green Fast	96.1	100

Table 1. Successful Amplification Using PrimeTime® qPCR Primers with Commercial Master Mixes. Randomly selected PrimeTime qPCR Primer sets (n=15) were tested with 5 popular commercial qPCR master mixes using the reaction set up described in Figure 1 with manufacturers' recommended cycling conditions. The data demonstrate >96% mean reaction efficiency for each of these master mixes.

PrimeTime® qPCR Primers

How to Order

PrimeTime qPCR Primers are available in Standard Size, premixed and normalized to 5 nmoles, providing enough primers for 500 20-µL reactions. Primer sequence is provided upon order, and Primer assays ship in 2–3 business days.



Order PrimeTime Primers through the PrimeTime qPCR Assay Selection Tool or RealTime PCR Tool on the IDT website (www.idtdna.com).