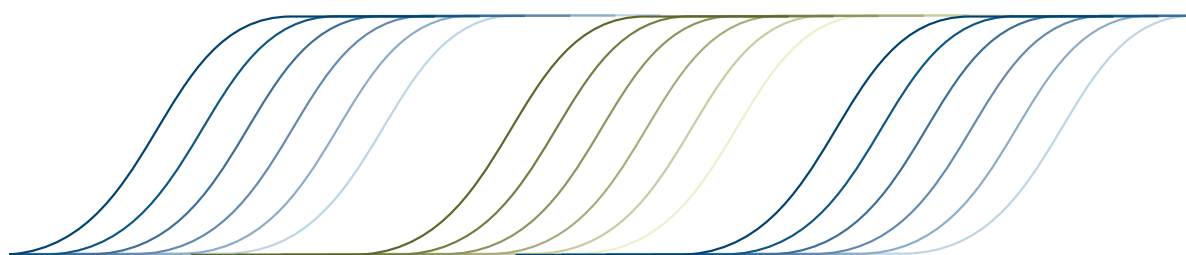# PrimeTime® Pre-designed qPCR Assays

5' nuclease assays for human, mouse, and rat

PrimeTime qPCR Assays

IDT now offers PrimeTime Pre-designed qPCR Assays that are guaranteed to work for human, mouse, and rat transcriptomes! PrimeTime qPCR Assays consist of two primers and a hydrolysis probe. All three components are combined into a single tube and shipped in 2–4 business days. Each oligo undergoes 100% QC by mass spectrometry, with all QC results provided free of charge on the IDT website.

Performance Guaranteed

- PrimeTime Pre-designed qPCR Assays are guaranteed to provide >90% efficiency when using Assays generated from the IDT Pre-designed Assay collection, a commercially available master mix, and measured over a minimum of four orders of magnitude.

- Users routinely experience lower C_q values, higher qPCR efficiency, and larger ΔRn with PrimeTime Pre-designed qPCR Assays than with competitor products.

Ultimate Flexibility

- Select from five different dye/quencher combinations and choose a premixed primer:probe ratio from 1:1 to 4:1 to maximize experimental flexibility and multiplexing capabilities.

- Choose from three different scales to best fit your experimental needs and reduce the number of unused reactions.

Easy Online Ordering

- Filter and sort by assay characteristics like exon location, splice variant targets, RefSeq number, or gene symbol.

- The ordering system contains Assays designed to target exon boundaries for every human, mouse, and rat transcript in the NCBI database.

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PrimeTime® Pre-designed qPCR Assays

Design Process

IDT's design engine incorporates many parameters to produce the best possible PrimeTime Pre-designed qPCR Assay. The design process uses the most current, clean, and complete target sequence information and stresses accurate T_m prediction and protection against off-target amplification. The process includes a wealth of additional secondary design considerations and design scoring criteria focused on isolating the best performance potential for a specific target design.

Access the most current sequence data available

Design assays with accurate T_m for primers and probes

Prevent cross reaction and off-target amplification, mask SNPs

Score remaining assays and publish to web

Current target sequence information

• The IDT design engine runs against the cleanest possible target data that include up-to-date SNP and intron/exon junction locations.

• The design libraries are continually refreshed as new sequence data become available.

Melting temperature (T_m)

• Accurate prediction of T_m is essential for precise PCR performance.

• The assay design engine incorporates the most currently available T_m prediction algorithms and nearest-neighbor parameter sets.

• IDT continues innovative DNA thermodynamics research and integrates those improvements into its calculation and oligonucleotide design tools.

Cross reaction and off-target amplification prevention

• Proprietary assay design engine algorithms and BLAST alignments check the target sequence to protect against cross reaction and off-target amplification.

• The algorithms reduce potential off-target amplicon formation, as well as individual element non-specificity that can compromise efficiency by consuming assay components through off-target interactions.

• The Assay design engine ensures that primers or probes are not placed on SNP locations.

Scoring and Publishing

The main assay design engine algorithm is tuned to aggressively "over-design". This produces dozens of possible assay options for each target which are then carefully weighed for selection of those with the best overall performance and reliability characteristics. These selection criteria include examination of characteristics such as poly runs, short sequence repeats, and hetero-dimer and homo-dimer folding energies.

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Validation of the Design Engine

To validate the design parameters, IDT tested 150 randomly selected PrimeTime Pre-designed qPCR Assays. Each assay was analyzed with a 5 log dilution series from 50 ng to 5 pg UHR cDNA. Efficiency measurements were calculated from the standard curve. All experiments were performed on the ABI 7900HT Fast Real-Time PCR System with the Applied Biosystems Gene Expression Master Mix.

Result Highlights

- More than 99% of the Assays tested exhibited >90% efficiency.

- 100% of the Assays had R^2 values >0.99.

- The mean efficiency of all Assays was 94.5%.

- Over 2/3 of all Assays had >95% efficiency.

Figure 1. IDT Assay Selection Engine Generates Highly Efficient qPCR Assays. 150 Assays were selected randomly and analyzed with a 5-fold standard curve of UHR cDNA. Each reaction was tested with 500 nM of each primer and 250 nM FAM/ZEN/IBFQ probe with the Applied Biosystems TaqMan® Gene Expression Master Mix. The reactions were run on the ABI 7900HT Fast Real-Time PCR System with the following PCR cycling conditions: 2 min. 50℃; 10 min. 95℃; 45 x (15 sec. 95℃, 1 min. 60℃).
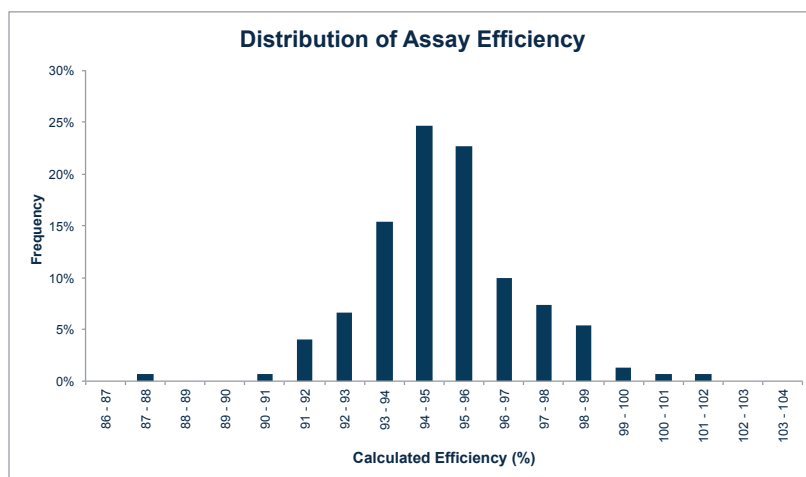
Pre-designed Assays Work With All Master Mixes Tested

16 different PrimeTime Pre-designed qPCR Assays were tested with 6 commercially available master mixes. A miniGene plasmid was used as a template and diluted over 6 logs to calculate efficiency. The reactions were all run on the ABI 7900HT instrument at the recommended concentration and cycling conditions for each master mix. All Assays exhibited greater than 97% efficiency with a 100% success rate.

Commercial Master Mix	Mean Efficiency (%)	Percent Success
Agilent Brilliant III Ultra Fast QPCR	96.89	100
Applied Biosystems TaqMan® Gene Expression	97.96	100
Invitrogen SuperMix	97.68	100
Qiagen QuantiTect® Probe PCR Kit	97.31	100
BioRad iTaq™ Supermix with ROX	100.52	100
BioSciences™ PerfeCTa™ qPCR	97.64	100

# PrimeTime® Pre-designed Assays Outperform Competitor Products

Performance Highlights

- **Improved Results for Fast Cycling** – With PrimeTime Pre-designed qPCR Assays and Agilent's Brilliant III Master Mix, you no longer have to sacrifice performance for speed.

- **Increased Sensitivity** – On average, PrimeTime Pre-designed qPCR Assays were nearly one full C_q lower and had endpoint signals more than 30% greater than the competition. (Figures 2 and 3).

- **Higher qPCR Efficiency** – When compared to Competitor A, not only did PrimeTime Pre-designed Assays have a higher average efficiency but the distribution of calculated efficiencies was also greatly reduced. (Figure 4).

PrimeTime Pre-designed Assays Excel with Fast-Cycling Protocols

Fast cycling allows for higher throughput and faster access to results. Unfortunately, researchers often have to sacrifice performance for speed. 6 PrimeTime Pre-designed qPCR Assays were tested using the Agilent Brilliant III Ultra Fast QPCR Master Mix, which allows run times as short as 45 minutes. These results were compared to 6 matched, inventoried assays from Competitor A.

- **No sacrifice in efficiency.** All PrimeTime Assays maintained efficiencies between 90–100%. 2 of the 6 Competitor A assays had efficiency values <90%.

- **Greater sensitivity.** PrimeTime Pre-designed qPCR Assays had lower C_q values compared to matched, inventoried assays from Competitor A by over 0.5 on average and ΔRn values that were almost 20% higher.
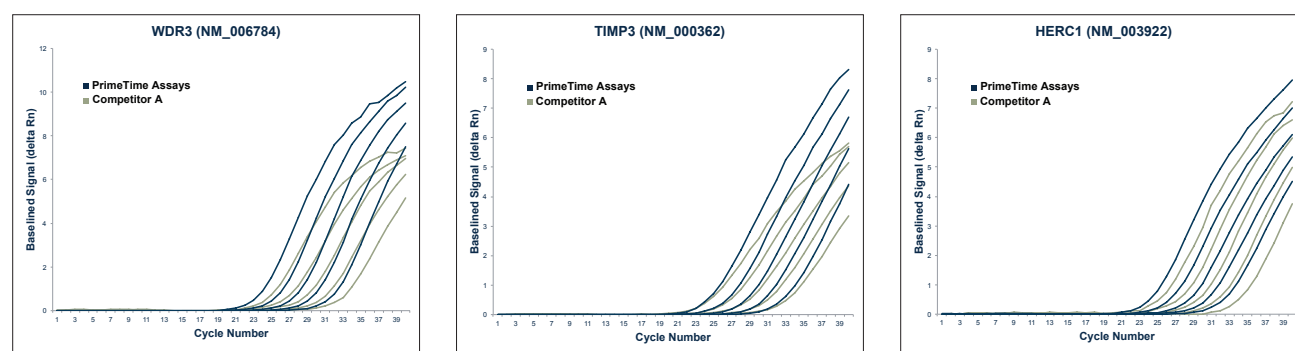
		WDR3	TIMP3	HERC1
Mean C_q (50 ng)	PrimeTime	22.9	23.9	25.1
	Competitor A	24.0	24.1	26.2
Efficiency	PrimeTime	97.0%	95.0%	95.9%
	Competitor A	96.4%	91.7%	86.8%
Endpoint (ΔRn)	PrimeTime	10.9	8.9	7.9
	Competitor A	7.2	6.1	7.0

Figure 2. PrimeTime Pre-designed qPCR Assays Maintain Efficiency Under Fast-Cycling Conditions. 6 PrimeTime Pre-designed qPCR Assays were compared to equivalent Competitor A assays designed to span the same exon boundaries. The reactions used 5-fold dilutions of cDNA template (miniGenes, IDT) and Brilliant III Ultra Fast QPCR Master Mix (Agilent) and were run on the ABI 7900HT Fast Real-Time PCR System with the following PCR cycling conditions: 3 min. 95°C; 45 x (5 sec. 95°C, 15 sec. 60°C). Identical thresholds were set for comparison across assays. 3 matched assays are shown: WDR3 (NM_006784), TIMP3 (NM_000362), and HERC1 (NM_003922). For each of the parameters shown (mean C_q, efficiency, and endpoint data), the PrimeTime Pre-designed qPCR Assays outperformed the Competitor A assays.

Increased Sensitivity

25 assays from Competitor A were compared to an equal number of IDT's PrimeTime Pre-designed qPCR Assays. The Competitor A assays consisted of 15 inventoried pre-designed assays and 10 made-to-order pre-designed assays. To ensure an accurate comparison was made, the PrimeTime Assays and Competitor A assays were selected to span the same exon boundary of each gene. The reactions were run with the Applied Biosystems Gene Expression Master Mix and identical thresholds were set for all runs (Figures 3 and 4).
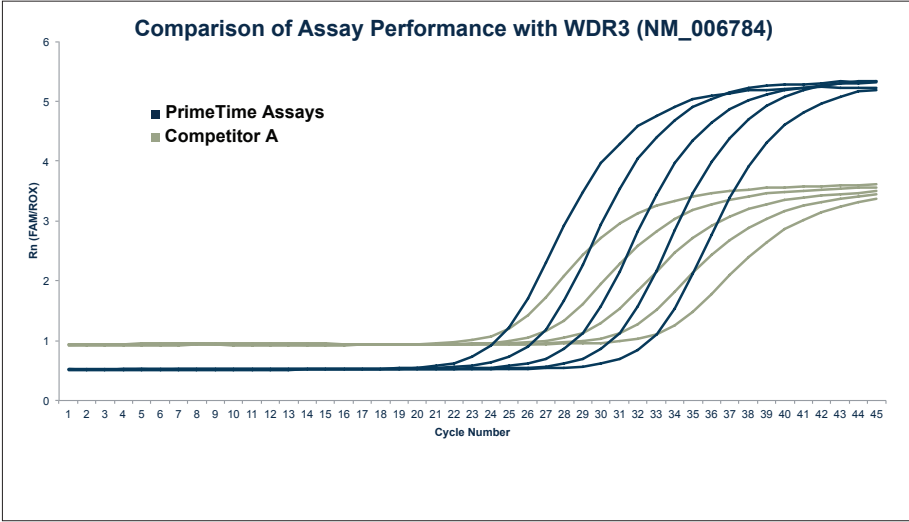
Figure 3. PrimeTime Assays Are More Sensitive than Competitor A Assays. PrimeTime Pre-designed qPCR Assays were compared to equivalent Competitor A assays using 5-fold dilutions of cDNA template and the Applied Biosystems TaqMan® Gene Expression Master Mix. The reactions were run on the ABI 7900HT Fast Real-Time PCR System with the following PCR cycling conditions: 2 min. 50°C; 10 min. 95°C; 45 x (15 sec. 95°C, 1 min. 60°C). Identical thresholds were set for all runs for comparison across assays. A comparison of the Competitor A WDR3 (NM_006784) assay and the equivalent IDT PrimeTime Pre-designed qPCR Assay is shown.
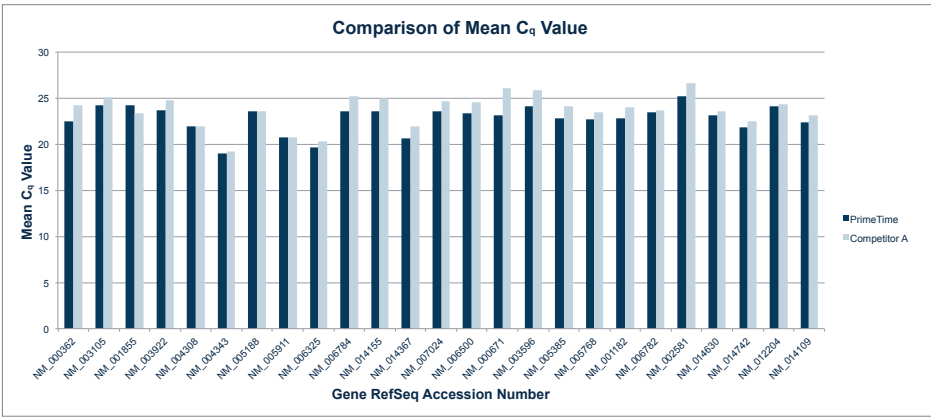
Figure 4. PrimeTime Pre-designed qPCR Assays Have Consistently Lower C_q Values for the Same Target. PrimeTime Pre-designed qPCR Assays were compared to equivalent Competitor A assays using 5-fold dilutions of UHR cDNA and the Applied Biosystems TaqMan® Gene Expression Master Mix. The reactions were run on the ABI 7900HT Fast Real-Time PCR System with the following PCR cycling conditions: 2 min. 50°C; 10 min. 95°C; 45 x (15 sec. 95°C, 1 min. 60°C). Identical thresholds were set for all runs for comparison across assays. The mean C_q values from the 50 ng dilution of UHR cDNA are shown.

*Successful assays were considered to be those that demonstrated >90% efficiency when measured over 5 logs with R_2 values >0.99.

Higher qPCR Efficiency

qPCR efficiency was measured using a comparison of 25 Competitor A and IDT assays for sensitivity (Figure 5). Again, the PrimeTime Pre-designed qPCR Assays have a higher average qPCR efficiency than Competitor A assays. In addition, the overall distribution of qPCR efficiency was narrower and higher than that for Competitor A assays.

Figure 5. PrimeTime Pre-designed qPCR Assays Have Higher qPCR Efficiency and a Smaller Distribution Range than Competitor A Assays. PrimeTime qPCR Assays were compared to matched Competitor A assays using 5-fold dilutions of cDNA and the Applied Biosystems TaqMan® Gene Expression Master Mix. The reactions were run on the ABI 7900HT Fast Real-Time PCR System with the following PCR cycling conditions: 2 min. 50°C; 10 min. 95°C; 45 x (15 sec. 95°C, 1 min. 60°C). Identical thresholds were set for all runs for comparison across assays.
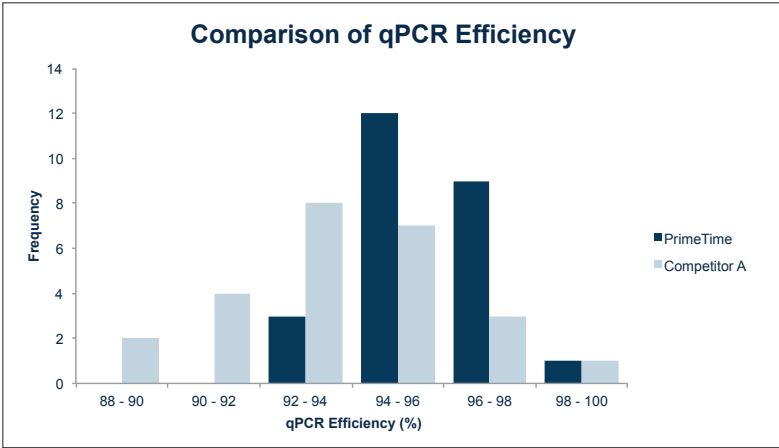
Ordering PrimeTime® Pre-designed Assays

1. Enter the gene name, RefSeq number, or Assay ID.

2. Select the species from mouse, human, or rat.

3. Click on *Submit*.

4. To sort the results, drag the column that you would like to sort by to the top. Drag more than one column to the top to further refine the search.

5. Select the Assay you would like to order.

6. Click on *Customize* to select the size (Mini, Standard, or XL), the dye-quencher combination, and the primer-to-probe ratio.

7. Click *Add To Order* or *Add And Checkout* button.

Enhanced Selection Features

A. Group by symbol, species, or splice variant by dragging the column title to the indicated space.

B. Filter by species, gene symbol, or splice variant.

C. Sort results by recommended Assays. When this is selected, the first Assay in the list will be the highest ranked and most recommended based on IDT scoring parameters.

D. View RefSeq numbers detected for each Assay design