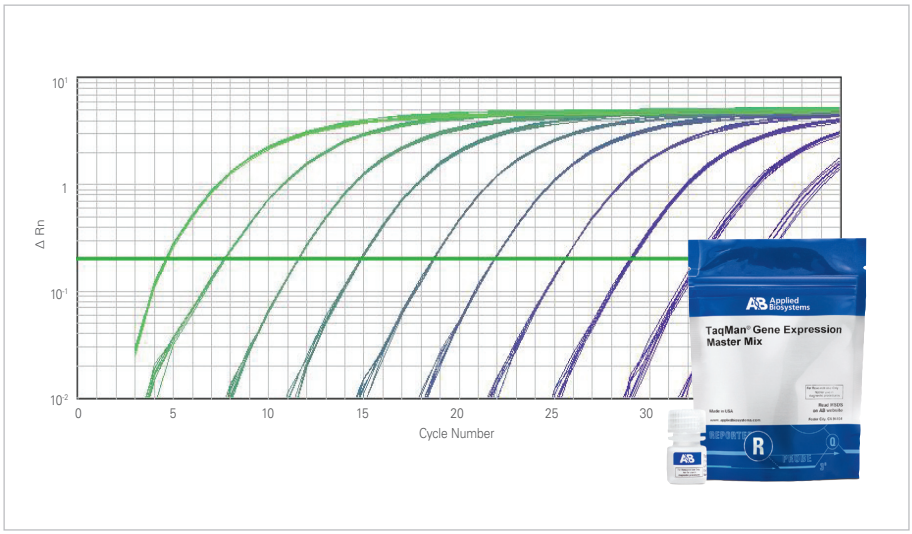
TaqMan® Gene Expression Master Mix and TaqMan® RNA-to-C_T™ *2-Step* Kit

Real-time PCR reagents tailored for quantitative PCR

Tailored for quantitative, real-time PCR experiments. Unrivaled sensitivity for both routine and challenging applications, rare transcript detection, duplex PCR, and specific detection of homologous sequences.

- Gene expression analysis
- Validation of RNAi-induced gene knockdown & microarrays
- Pathogen detection & viral load quantification



Introduction

TaqMan® Gene Expression Master Mix delivers sensitive and specific detection across a broad range of template quantities, down to a single copy of target. For precise and consistent quantification, the mix can detect a target and a reference gene in duplex PCR, and quantify less than two-fold differences in target amounts. For specific detection, the mix offers discrimination between homologous sequences, such as gene family members. For ease of use, TaqMan Gene Expression Master Mix uses universal thermal cycling conditions and can replace TaqMan® Universal PCR Master Mix in existing protocols.

Benefits

- Sensitive detection
  - for reliable quantification of abundant and limited targets
  - for clear discrimination between similar quantities of targets
- Duplex PCR for co-amplifying two targets in a single reaction
- Specificity for differentiation between gene family members
- Stable mix for high-throughput handling
- Validated with TaqMan® Gene Expression Assays for exceptional performance

Optimized Formulation for Unrivaled Performance

TaqMan Gene Expression Master Mix is a convenient 2X mix for target quantification that includes:

- AmpliTaq Gold® DNA Polymerase, UP (Ultra Pure), a highly purified DNA polymerase for improved detection of bacterial targets. This hot-start enzyme is inactive at room temperature so reactions can be set up on the benchtop. Enzyme is activated during thermal cycling.
- Uracil-DNA Glycosylase (UDG) to minimize carryover PCR contamination.
- Passive internal reference based on proprietary ROX™ dye for precise data analysis.

Reliable Quantification of Abundant and Limited Targets

TaqMan® Gene Expression Master Mix provides dependable target quantification over a wide dynamic range. The amplification of a dilution series of synthetic target sequence shows excellent PCR efficiency across nine orders of magnitude of template quantities using TaqMan Gene Expression Master Mix (Figure 1).

The sensitivity of TaqMan Gene Expression Master Mix was validated using a single-copy gene, RNase P, amplified from low amounts of human genomic DNA (gDNA). Since significant sampling error occurs when measuring low quantities of target, proper evaluation requires statistical analysis of multiple replicates. Figure 2 shows the expected quantity of target and corresponding mean C_T values. Statistical analysis indicates high confidence of sample quantification based on a T-test (Table 1), consistent with single-copy detection of target. TaqMan Gene Expression Master Mix enables detection of small quantities of target, such as transcripts expressed at low levels. For the most consistent detection, use with TaqMan® PreAmp Master Mix to detect targets in precious samples.

Discrimination Between Similar Abundance Levels

The sensitivity provided by TaqMan Gene Expression Master Mix facilitates quantifying small differences (less than 2-fold) in target amount between samples. For example, the amplification plots in Figure 3 show clear, statistically significant discrimination at 1.5-fold differences between samples of RNase P gene amplified from 0.66 and 0.99 ng gDNA, which have approximately 200 and 300 copies of the RNase P target, respectively.

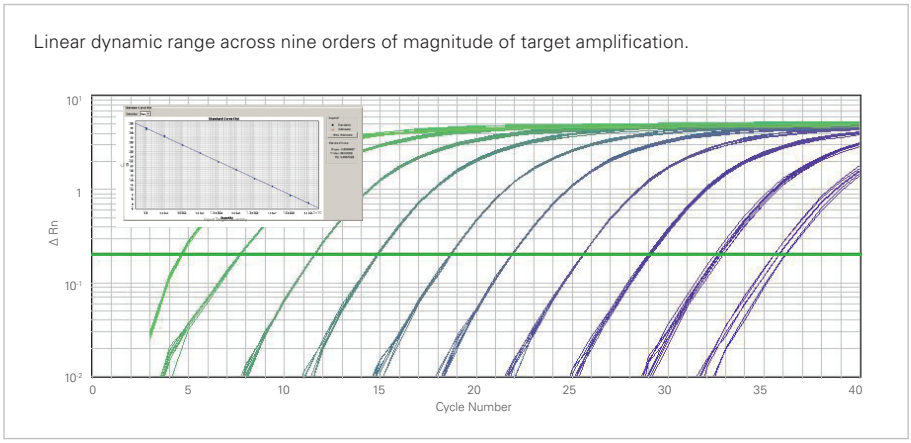


Figure 1. Amplification plot and standard curve of a synthetic target amplified from a dilution series of template in eight replicate reactions using TaqMan® Gene Expression Master Mix on the Applied Biosystems 7900HT Fast Real-Time PCR System.

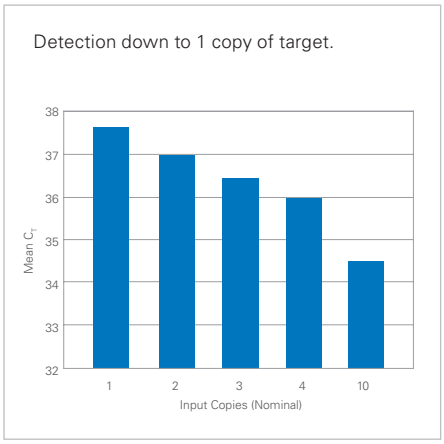


Figure 2. RNase P target amplified from 3.3–33 pg of human gDNA, which corresponds to ~1–10 copies of target, in 64 replicate reactions using the Applied Biosystems 7900HT Fast Real-Time PCR System.

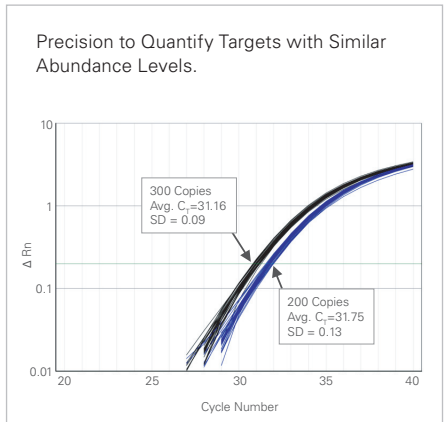


Figure 3. Discrimination between 200 and 300 copies of RNase P gene, amplified from human gDNA (32 replicate reactions) on the Applied Biosystems 7500 Real-Time PCR System, with 99.7% confidence.

TABLE 1. Statistical T-test to evaluate detection of small amounts of target

Nominal Copies	N†	Mean C_T	t-Value	p-Value	Confidence‡
1	44	37.63	4.15	<0.0001	99.9%
2	61	36.97			
2	61	36.97	3.75	<0.0001	99.9%
3	63	36.44			
3	63	36.44	3.33	0.0006	99.9%
4	64	35.97			
4	64	35.97	11.81	0	99.9%
10	64	34.52			

†Number of replicates out of 64 replicate reactions with C_T <40. Due to sampling error, the Poisson distribution predicts that some samples with very few targets per reaction contain zero copies of target. These results closely match the Poisson distribution.

‡ Confidence is the probability that the mean C_T of samples with fewer input copies of target is greater than the mean C_T of samples that contained more input copies of target.

Consistent Performance for Single-Target and Duplex Real-Time PCR.

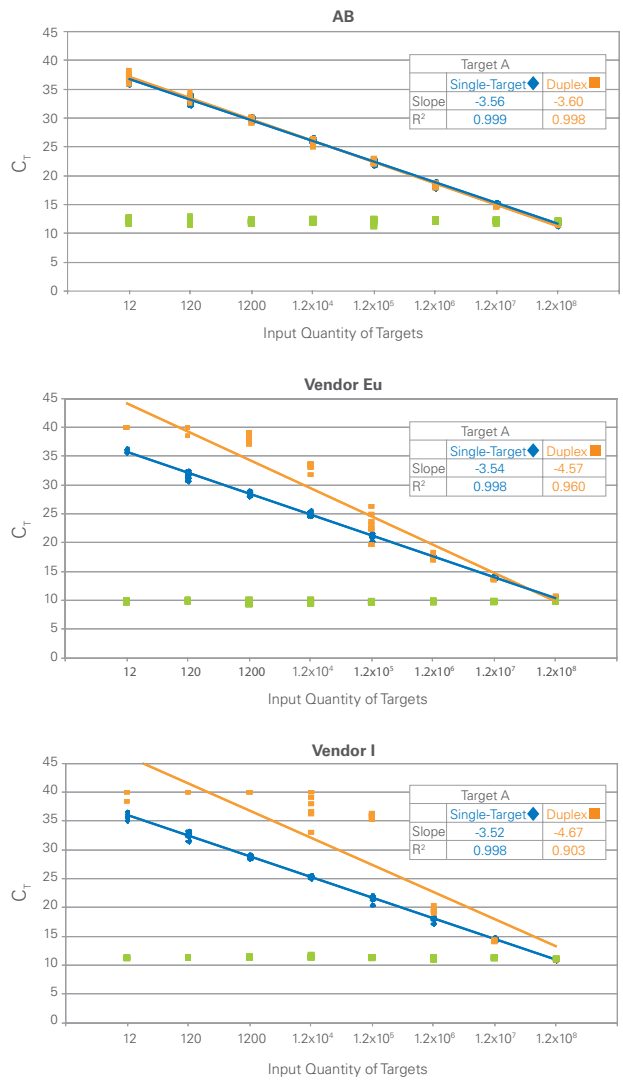


Figure 4. Experimental target A (FAM™) amplified in single-target (blue diamonds) and duplex reactions (orange squares) with reference target B (VIC®, green squares). TaqMan® Gene Expression Master Mix (AB) and other commercial mixes (Eu, I) were used. Reactions (eight replicates) were run on the Applied Biosystems 7900HT Fast Real-Time PCR System. A C_T of 40 was assigned to samples that failed to amplify after 40 cycles.

Robust Results for Duplex PCR

Co-amplification, or duplex PCR, simultaneously amplifies two targets in a single tube to allow for increased sample throughput. The following series of reactions compare the performance of TaqMan® Gene Expression Master Mix to other commercial mixes. The experimental target, synthetic target A, was serially diluted and amplified in single-target reactions and in duplex reactions. The duplex reactions include target A and a constant quantity of a synthetic reference target B (primer-limited conditions for the reference target). TaqMan Gene Expression Master Mix succeeds in showing comparable results for experimental target A between single-target and duplex reactions. Both sets of reactions exhibit high PCR efficiencies across the range of target quantities tested (Figure 4). By contrast, mixes from Vendors Eu and I, run under identical reaction conditions, fail to amplify low quantities of experimental target A in duplex reactions containing reference target B.

Targets with GC- or AT-rich regions may be challenging to amplify efficiently due to design constraints. GC- or AT-rich targets were amplified in duplex PCR from a range of cDNA quantities with either TaqMan Gene Expression Master Mix or a commercial mix from Vendor R (Figure 5). TaqMan Gene Expression Master Mix shows a linear response to target dilution with excellent PCR efficiencies. Vendor R mix shows poor amplification efficiency for these challenging targets. For quantification of AT- and GC-rich targets, TaqMan Gene Expression Master Mix meets the challenge.

Specificity for Differentiation of Similar Sequences

Specific target detection is a key concern when studying gene family members, viruses, and pathogens, where a sample may contain several similar sequences. Specificity was tested in a model system to compare TaqMan Gene Expression Master Mix and 11 other mixes. The model system used a target based on a member of a large family of ATP-binding cassette proteins (ABCB10). The target was amplified from two synthetic templates consisting of a perfect-match sequence and a sequence with a few mismatches. TaqMan Gene Expression Master Mix preferentially detected the perfect-match template with greater than 8000-fold discrimination compared to the related sequence. TaqMan Gene Expression Master Mix provides the highest specificity of 12 mixes tested (Figure 6).

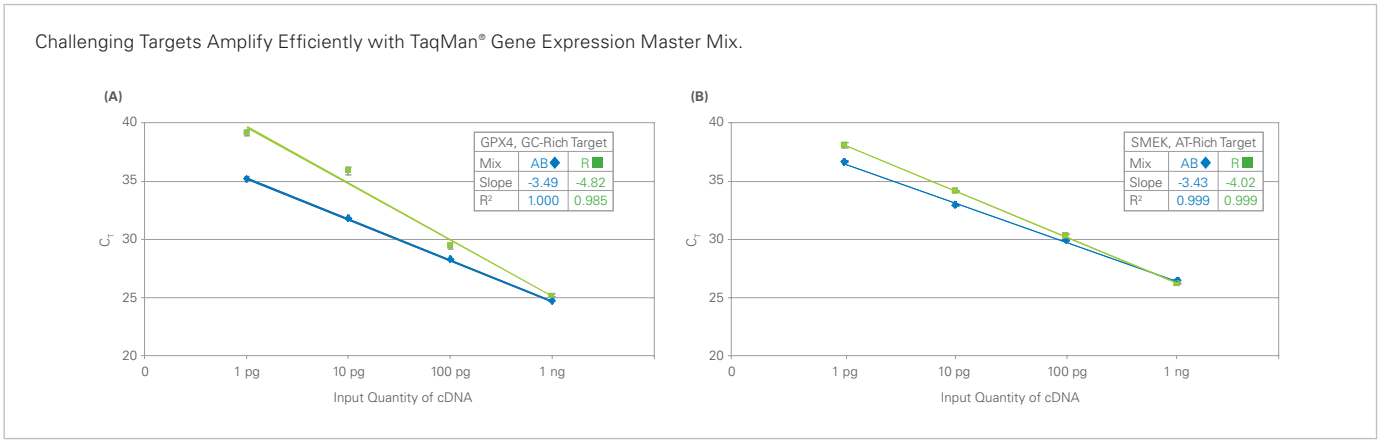


Figure 5. Serial dilutions of cDNA were utilized as template in eight replicate duplex PCRs (data not shown for 18S reference), using either TaqMan® Gene Expression Master Mix (AB, dark blue) or mix from Vendor R (green). Standard curves generated from amplification of GPX4, a target with GC-rich regions (A), and SMEK2, a target with AT-rich regions (B). Reactions were performed on the Applied Biosystems 7900HT Fast Real-Time PCR System.

Stable Mix for High-Throughput Handling

Extended benchtop stability of assembled reaction mixtures provides flexibility to process numerous samples at room temperature. The stability of TaqMan® Gene Expression Master Mix was demonstrated in 48 duplex PCRs amplifying an experimental target and a reference target, 18S rRNA. PCR was performed immediately (0 hours), or following storage for 72 hours. All 48 targets and reference target show equivalent amplification curves for the time points tested (Figure 7 shows a subset of targets). Even after 72 hours, the excellent stability of TaqMan Gene Expression Master Mix provides accurate and consistent results for automated liquid handling systems used in high throughput sample processing.

TaqMan® RNA-to-C_T™ *2-Step* Kit

The TaqMan RNA-to-C_T *2-Step* Kit, a combination of the High Capacity RNA-to-cDNA Kit and the TaqMan Gene Expression Master Mix, provides dependable and sensitive target quantification over a wide dynamic range. The High Capacity RNA-to-cDNA Kit is used to first reverse transcribe RNA into cDNA and the resulting cDNA is then quantitated using the TaqMan RNA-to-C_T *2-Step* Kit for real-time PCR.

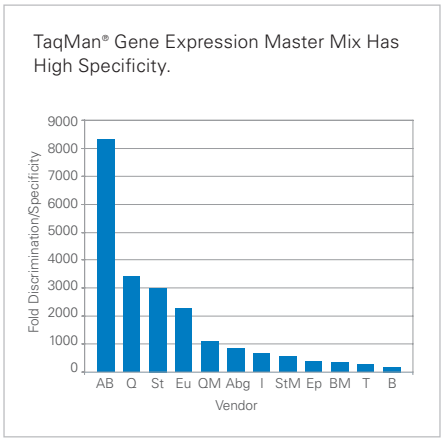


Figure 6. Specific detection of ABCB10 (TaqMan® Gene Expression Assay) over a similar sequence containing mismatches (24 replicate reactions), by different mixes using the Applied Biosystems 7900HT Fast Real-Time PCR System. The Y-axis shows the fold discrimination between the perfect match target compared to the mismatched target $2^{\Delta C_T}$.

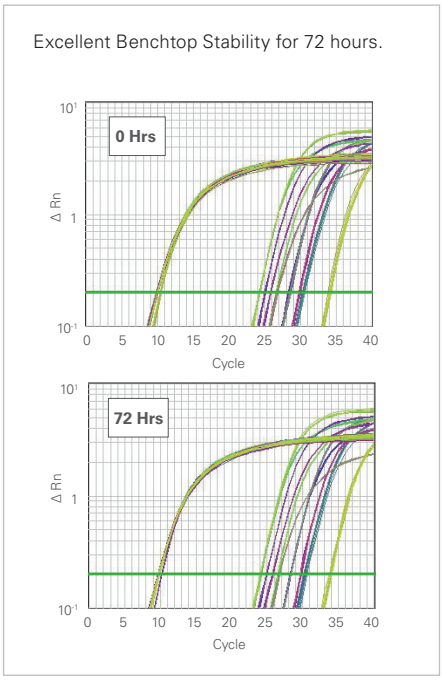


Figure 7. Targets amplified from cDNA in duplex PCRs, immediately after reaction assembly or after storage for 72 hours, using TaqMan® Gene Expression Master Mix on the Applied Biosystems 7900HT Fast Real-Time PCR System. Amplification curves of 11 representative targets (CCDC125, MGC16384, SC4MOL, RREB1, GPR21, ALAS1, RNF38, ZBTB10, CTCF, ZNF613, CTNNA3) and the reference target 18S are shown (eight replicates each).

TaqMan® Gene Expression Master Mix Compatibility Chart	
Instruments, Assays and Reagents	**Compatibility Data (standard thermal cycling mode)**
StepOne™ Real-Time PCR System	Yes
StepOnePlus™ Real-Time PCR System	Yes
Applied Biosystems 7300 Real-Time PCR System	Yes
Applied Biosystems 7500 Real-Time PCR System	Yes
Applied Biosystems 7500 Fast Real-Time PCR System	Yes
Applied Biosystems 7900HT Fast Real-Time PCR System	Yes
ABI PRISM® 7000 Sequence Detection System	Yes
TaqMan® Gene Expression Assays	Yes
TaqMan® Arrays	Yes
High Capacity RNA-to-cDNA Kit	Yes
High Capacity RNA-to-cDNA Master Mix	Yes

Notes

ORDERING INFORMATION

Description	Quantity	Reactions†	Part Number
TaqMan® Gene Expression Master Mix			
Mini-Pack	1 mL tube	40 PCR	4370048
1-Pack	5 mL bottle	200 PCR	4369016
2-Pack	2 x 5 mL bottle	400 PCR	4369514
5-Pack	5 x 5 mL bottle	1,000 PCR	4369510
10-Pack	10 x 5 mL bottle	2,000 PCR	4369542
Bulk Pack	50 mL bottle	2,000 PCR	4370074
Quick Reference Card 4371134	1 card	—	
Protocol 4371135	1 protocol	—	
TaqMan® RNA-to-C_T™ *2-Step* Kit‡			
Mini-Pack	1 tube 50 µL 20x RT Enzyme Mix 1 tube 500 µL 2x RT Buffer Mix 1 tube 1 mL TaqMan® Gene Expression Master Mix	50 RT 40 PCR	4399902
1-Pack	1 tube 50 µL 20x RT Enzyme Mix 1 tube 500 µL 2x RT Buffer Mix 1 bottle 5 mL TaqMan® Gene Expression Master Mix	50 RT 200 PCR	4399367

†assume 50 µL reaction volume for PCR and 20 µL reaction volume for RT

‡includes TaqMan Gene Expression Master Mix and High Capacity RNA-to-cDNA Kit

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NOTICE TO PURCHASER: LIMITED LICENSE
Practice of the patented 5′ Nuclease Process requires a license from Applied Biosystems. The purchase of the TaqMan® Gene Expression Master Mix and TaqMan® RNA-to-C_T™ *2-Step* Kit includes an immunity from suit under patents specified in the product insert to use only the amount purchased for the purchaser's own internal research when used with the separate purchase of Licensed Probe. No other patent rights are conveyed expressly, by implication, or by estoppel. Further information on purchasing licenses may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Purchase of the TaqMan® Gene Expression Master Mix is accompanied by a limited license under U.S. patents and foreign equivalents to use for research.

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