TaqMan® Universal Master Mix II

The Real-Time PCR Master Mix for Multiple TaqMan® Applications

Optimized formulation for all quantitative real-time PCR applications:

- Gene expression analysis
- MicroRNA analysis
- SNP genotyping
- Copy number genotyping
- Validation of RNAi-induced gene knockdown and microarray results
- Pathogen detection and viral load quantification

Introduction

TaqMan® Universal Master Mix II brings the quality and reliability you've come to expect from Applied Biosystems to a new, improved master mix formulation for real-time PCR. Whether your experiments require sensitivity and precision across a broad range of input target quantities, reliable detection of low copy number targets, or accurate quantification to discriminate subtle differences in target abundance, TaqMan® Universal Master Mix II fits your requirements. Like all TaqMan®-based technologies, the master mix offers single-base discrimination between homologous sequences, and reactions can be run using universal thermal cycling conditions. Finally, TaqMan® Universal Master Mix II can be directly substituted into your existing protocols.

Benefits

- Stable at room temperature for 24 hours in a pre-assembled PCR reaction
- Validated with Applied Biosystems TaqMan® Assays for Gene Expression, SNP Genotyping, and MicroRNA
- Uses universal thermal cycling conditions for TaqMan® Assays

Everything You Need for Everyday Real-Time PCR

TaqMan® Universal Master Mix II is a convenient 2X mix that includes:

- AmpliTaq Gold® DNA Polymerase, UP (Ultra Pure), a highly purified thermostable DNA polymerase that is hot start–enabled for convenient reaction setup at room temperature and improved detection of bacterial targets
- dNTP mixture: the master mix is available in two formats: with and without uracil-DNA glycosylase (UDG) and dUTP/dTTP mix to minimize carryover PCR contamination
- Optimized salt, dNTP, and buffer concentrations for reliable performance
- Passive internal reference based on proprietary ROX™ dye for precise data analysis

Sensitivity and Wide Dynamic Range for Expression Analysis

TaqMan® Universal Master Mix II provides dependable target quantification over a wide dynamic range of expression levels, so that you can detect and quantitate high and low expressors in a single experiment. This is illustrated in Figure 1; a single-copy target, the RNase P gene, was amplified from a dilution

series of human DNA using TaqMan® Universal Master Mix II. The data demonstrate excellent PCR linearity over a 7-log range of input template. Figure 2 shows a similar experiment conducted using TaqMan® MicroRNA Assays. The amplification of a dilution series of a synthetic microRNA target sequence was linear across 8 orders of magnitude of template quantities using TaqMan® Universal Master Mix II (Figure 2).

Specificity to Distinguish Highly Homologous Targets

TaqMan® Universal Master Mix II also provides excellent specificity in real-time PCR. MicroRNAs are small (21–23 nt) nucleic acids that are involved in the regulation of many genes and have been implicated in disease processes such as cancer and heart disease [1, 2, 3, 4]. Some members of microRNA families differ from each other by as little as 1 nucleotide, presenting a difficult challenge for many master mixes. The TaqMan® Universal Master Mix II is formulated to deliver the necessary specificity to distinguish between these highly homologous targets (Table 1).

Benchtop Stability for High-Throughput Handling

TaqMan® Universal Master Mix II is formulated with high-quality components that retain the ability to discriminate between small relative differences in target concentration even after incubating assembled PCR reactions for 24 hours at room temperature (Figure 3). This extended benchtop stability provides flexibility to process numerous samples using high-throughput liquid handling systems that may require assembled reactions to sit at room temperature until they can be fed into the real-time PCR instrument. The stability of PCR reactions fueled with TaqMan® Universal Master Mix II is shown in Figure 3. In this experiment, PCR cycling was initiated immediately (0 hours) upon setup of the reactions, or after storage of assembled reactions at 30°C for 24 hours.

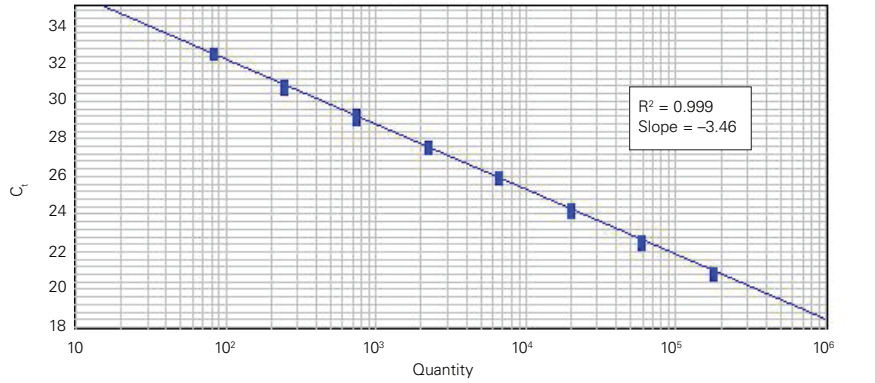

Figure 1. Consistent Real-Time PCR Results Across a 7-Log Dilution of Target Input. Amplification plot and standard curve from real-time PCR for a dilution series of human CEPH genomic DNA amplified in 6 replicate reactions using the Applied Biosystems 7900HT Fast Real-Time PCR System and human RNase P gene expression assay.

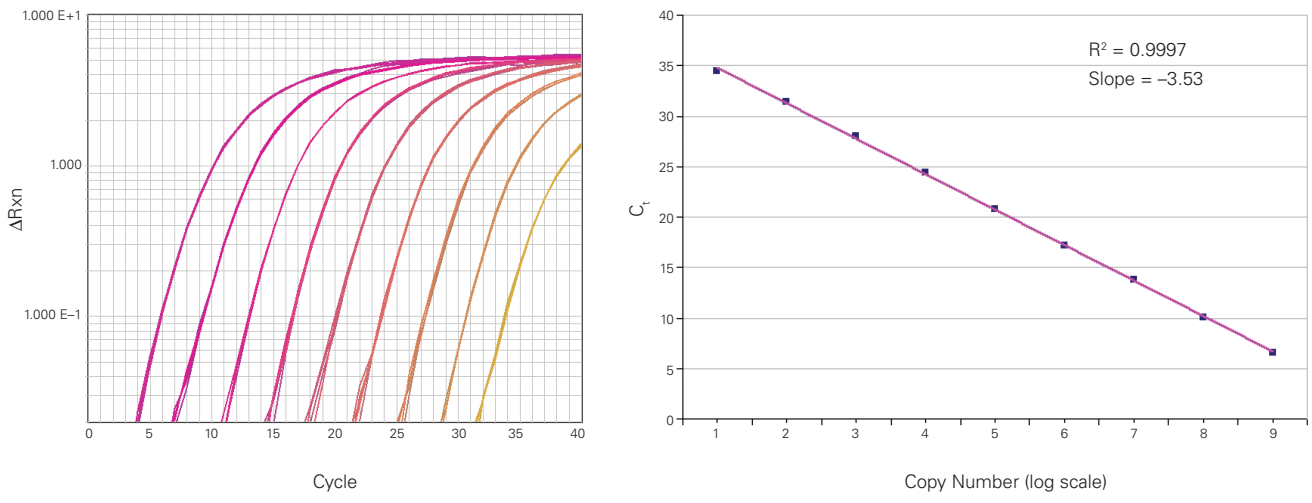

Figure 2. Linear Target Amplification With a Dynamic Range Across 8 Orders of Magnitude of Input. Amplification plot and standard curve from real-time PCR for a dilution series of a synthetic target amplified in 4 replicate reactions using the Applied Biosystems 7900HT Fast Real-Time PCR System and Let7-b TaqMan® MicroRNA Assay.

	TaqMan® MicroRNA Assay Used					
Template (0.5 pM)	Let7-a	Let7-b	Let7-c	Let7-d	Let7-e	Let7-f
Let7-a	1.00	0.00	0.00	0.00	0.00	0.03
Let7-b	0.00	1.00	0.03	0.00	0.00	0.00
Let7-c	0.00	0.03	1.00	0.00	0.00	0.00
Let7-d	0.00	0.00	0.00	1.00	0.00	0.00
Let7-e	0.00	0.00	0.00	0.00	1.00	0.00
Let7-f	0.06	0.00	0.00	0.00	0.00	1.00

Table 1. Minimal Cross-Amplification of Highly Homologous Members of the Let7 MicroRNA Family Using TaqMan® Universal Master Mix II. In this experiment, synthetic microRNAs representing the indicated members of the Let7 family of microRNAs, some of which differ by as little as a single nucleotide, were amplified using TaqMan® MicroRNA Assays for each member of the Let7 family. The table shows percent cross-amplification. In most cases, TaqMan® Universal Master Mix II and TaqMan® MicroRNA Assays amplified only the appropriate target. In the few cases where cross-amplification was seen (values highlighted in blue) it represented only a few percent of the PCR product amplified.

Validated for TaqMan® Genotyping Assays

TaqMan® Universal Master Mix II has been validated for use with genotyping assays. The formulation provides well-separated clusters for all categories of assays under universal cycling conditions. Figure 4 demonstrates excellent allelic discrimination in TaqMan® SNP Genotyping Assays run using TaqMan® Universal Master Mix II. In this experiment, 43 genomic DNA samples were queried using a TaqMan® Drug Metabolism Enzyme Assay following the recommended protocol. As the plot illustrates, data clusters are well separated, and samples could be easily called as homozygous or heterozygous.

TaqMan® Universal Master Mix II for Confidence in Real-Time PCR

TaqMan® Universal Master Mix II is formulated with ultrapure components to provide a 14-month shelf life. It is manufactured to provide maximum confidence in your results, with reduced bacterial DNA carryover, and more rigorous quality control and quality reporting than ever before. Even the packaging has been redesigned to minimize waste. TaqMan® Universal Master Mix II is the one mix you can count on for multiple real-time PCR applications. It has been extensively optimized, tested, and validated to provide clean, clear results for gene expression and microRNA quantitation, TaqMan® genotyping using SNP and copy number assays, pathogen detection and viral load quantification, and validation of microarray and RNAi-induced gene knockdown data.

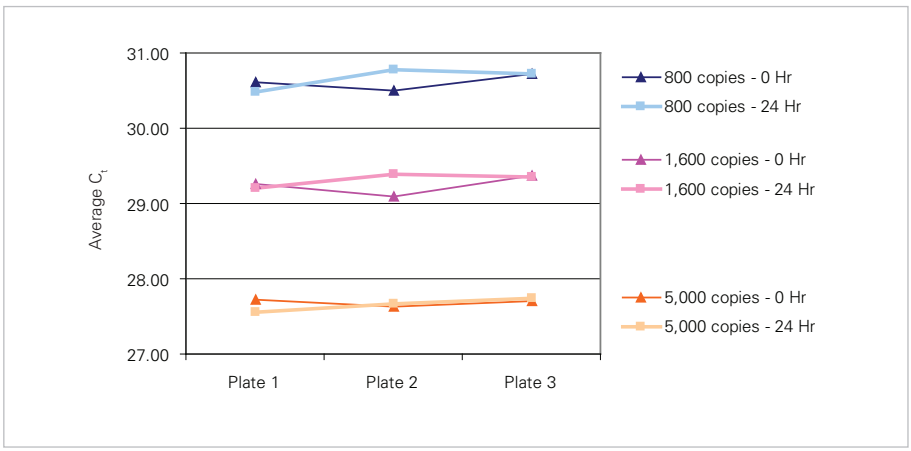

Figure 3. Equivalent C_t Values and Linear Target Detection Even After 24 Hr Room Temperature Incubation of Pre-Assembled PCR Reaction. The RNase P gene was amplified from human genomic DNA using TaqMan® Universal Master Mix II in reactions that were run either just after setup or after sitting at room temperature for 24 hr. The results for all dilutions are consistent across the 3-plate replicates regardless of whether reactions were subjected to thermal cycling immediately or left at room temperature before cycling.

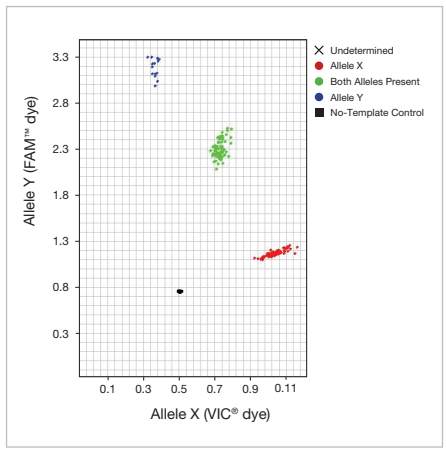

Figure 4. TaqMan® Universal Master Mix II Provides Excellent Genotyping Results. TaqMan® DME Assay C__26457248_10 was run using 43 gDNA samples with 4 replicates each in a 384-well format. These experiments were performed on the 7900HT Fast Real-Time PCR System from Applied Biosystems.

References

1. Lu J, Getz G, Miska EA et al. (2005) MicroRNA expression profiles classify human cancers. *Nature* **435(7043)**:834–838.

2. O'Donnell KA, Wentzel EA, Zeller KI et al. (2005) c-Myc-regulated microRNAs modulate E2F1 expression. *Nature* **435(7043)**:839–843.

3. Thum T, Galuppo P, Wolf C et al. (2007) MicroRNAs in the human heart: a clue to fetal gene reprogramming in heart failure. *Circulation* **116(3)**:258–267.

4. van Rooij E, Sutherland LB, Liu N et al. (2006) A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. *Proc Natl Acad Sci U S A* **103(48)**:18255–18260.

ORDERING INFORMATION

Product	Quantity	50 µL Reactions	P/N
TaqMan® Universal Master Mix II, no UNG			
Mini-Pack	1 mL tube	40	4440043
1-Pack	5 mL bottle	200	4440040
2-Pack	2 x 5 mL bottle	400	4440047
5-Pack	5 x 5 mL bottle	1,000	4440048
10-Pack	10 x 5 mL bottle	2,000	4440049
Bulk Pack	50 mL bottle	2,000	4440041
TaqMan® Universal Master Mix II, with UNG			
Mini-Pack	1 mL tube	40	4440042
1-Pack	5 mL bottle	200	4440038
2-Pack	2 x 5 mL bottle	400	4440044
5-Pack	5 x 5 mL bottle	1,000	4440045
10-Pack	10 x 5 mL bottle	2,000	4440046
Bulk Pack	50 mL bottle	2,000	4440039

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applied biosystems™

Headquarters
850 Lincoln Centre Drive | Foster City, CA 94404 USA
Phone 650.638.5800 | Toll Free 800.345.5224
www.appliedbiosystems.com

International Sales
For our office locations please call the division headquarters or refer to our website at
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