

TaqMan® Gene Expression Assays

Product Guide



Applied Biosystems TaqMan® Gene Expression Assays Solutions

Applied Biosystems offers the largest family of products to meet your quantitative gene expression needs: from off-the-shelf gene-specific probe and primer sets to Custom TaqMan® probes and primers manufactured to your desired sequences, and everything in between. All products use TaqMan® probe-based chemistry and are designed for use on the suite of Applied Biosystems Real-Time PCR Systems—
together the gold standard in quantitative gene expression offering the greatest sensitivity, specificity, reproducibility, and the broadest dynamic range.

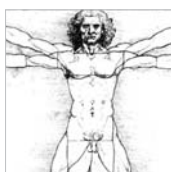
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Which TaqMan® Gene Expression Product Is Right for You?	TaqMan® Gene Expression Assays		Custom TaqMan® Gene Expression Assays	TaqMan® Endogenous Controls	Custom TaqMan® Probes and Primers
	Inventoried	Non-Inventoried			
Convenience of fully-formulated assay (premixed probe and primers)	✓	✓	✓	✓	
No specific preference on assay location within a gene	✓				
Specific location within a transcript is key		✓	✓		✓
FAM™ dye label is fine	✓	✓	✓	✓	✓
Need a specific reporter dye label				✓	✓
Prefer to use an Applied Biosystems design	✓	✓	✓	✓	
Prefer to use my own sequence target			✓		✓
Prefer to design my own probe and primers					✓
Non-universal probe-primer concentration					✓

TaqMan® Gene Expression Assays

- Gene-specific TaqMan® probe and primer sets for quantitative gene expression studies in human, mouse, rat, Arabidopsis, and Drosophila species
- Convenient single-tube format
- Universal cycling conditions

TaqMan® Gene Expression Assays are a comprehensive collection of over 600,000 pre-designed probe and primer sets that let researchers quickly and easily perform quantitative gene expression studies on human, mouse, rat, Arabidopsis, or Drosophila genes. Each gene expression assay consists of a FAM™ dye-labeled TaqMan® MGB probe and two PCR primers formulated into a single tube. Every assay is optimized to run under universal thermal cycling conditions with a final reaction concentration of 250 nM for the probe and 900 nM for each primer. This streamlined approach and comprehensive assay selection enables a convenient, standardized process for quantitative gene expression.



Human Assays

Over 100,000 gene expression assays are available for all known human genes. These include genes in the public domain with associated RefSeq transcripts (NCBI Reference Sequence project database: <http://www.ncbi.nlm.nih.gov/RefSeq>), the mammalian gene collection (MGC), and GenBank® database. A minimum of one assay (probe and primer set) per RefSeq transcript is available as an inventoried, off-the-shelf product currently numbering > 21,000 assays. The complete collection includes assays for nearly every exon junction in all known human genes in the public domain covering most probes on the Applied Biosystems Expression Array System.

Mouse and Rat Assays

Over 200,000 mouse and rat assays have been designed for all known genes. As with our human assays, at least one assay per RefSeq transcript has been manufactured and is available from our inventory. High quality assay designs for all other genes are also available on a made-to-order basis, as non-inventoried assays.

Strain-Neutral Mouse and Rat Assays

The assay design process yields strain-neutral mouse and rat gene expression assays. Polymorphisms are the cause of most sequence variability between

strains. By avoiding areas in the gene transcripts of known polymorphisms, we design only strain-neutral gene expression assays.

Arabidopsis and Drosophila Assays

Over 130,000 assays have been designed for most known genes for *Arabidopsis thaliana* and *Drosophila melanogaster*. Similar to the assays for other species, these assays have been designed against the public RefSeq database. The collection includes assays for nearly every known transcript for each organism, and all assays are available on a made-to-order basis.

Comprehensive Coverage and Selection

Not only have we designed an assay for every gene, but also for multiple locations across each gene transcript. More than 600,000 high-quality assay designs are available for human, mouse, rat, Arabidopsis, and Drosophila genes on a made-to-order basis. This vast selection allows researchers to select the specific location on a given transcript they wish to detect. For instance, microarray researchers that may prefer a 3' bias in their TaqMan® probe and primer sets will be able to select from robust, pre-designed TaqMan® Gene Expression Assays. Additionally, researchers performing RNAi studies can choose multiple assays per gene to validate their knockdown results.



TaqMan® Gene Expression Assays are delivered with a compact disc containing an electronic assay information file.

State-of-the-Art Assay Design Bioinformatics

All assays are designed using Applied Biosystems sophisticated bioinformatics pipeline, customized for either the human, mouse, rat, Arabidopsis, or Drosophila genome. This pipeline consists of three main steps:

Step One

Both public and Celera sequence data are used to identify the optimal probe and primer locations. This process consists of:

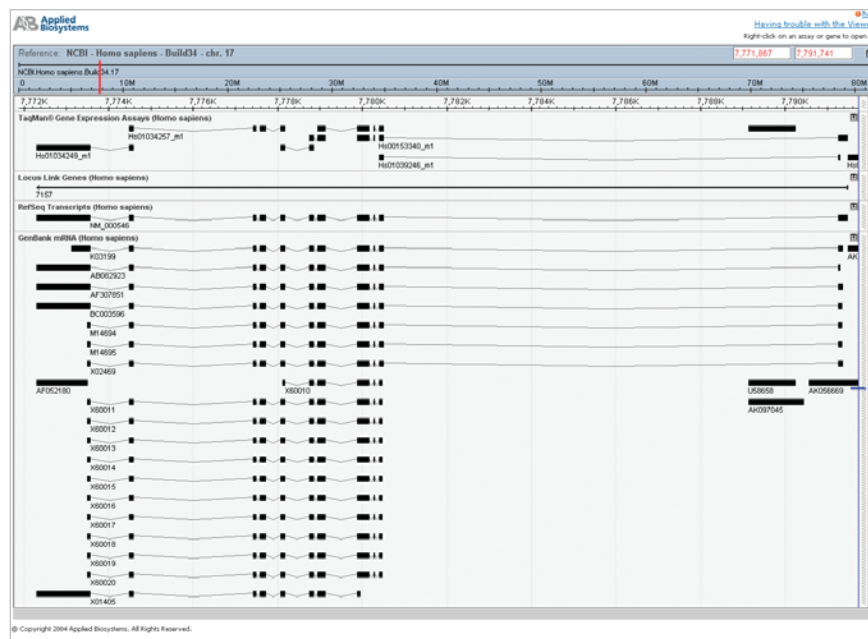
- Mapping transcripts to the genome to identify exon boundaries
- Masking sequence discrepancies between public and Celera data
- Masking sequence repeats
- Masking known SNPs from both public and Celera databases

Step Two

Proprietary software algorithms generate probe and primer designs for the locations identified above. These algorithms include optimal design parameters, such as %GC content, T_m , amplicon length, and low secondary structure, to ensure high amplification efficiency. Where possible, designs span an exon-exon junction, eliminating the possibility of detecting genomic DNA.

Step Three

In silico QC ensures each assay is specific to the gene for which it was designed (i.e., the assay will not detect sequences from other genes, or pseudo-genes). Each assay design is processed through a quality scoring system and one high scoring, gene-specific assay design is sent to our state-of-the-art manufacturing facility. All designs meeting our scoring criteria are also displayed in our online



The Applied Biosystems online catalog map view graphically represents the assay's physical location on the gene, and which transcript(s) each assay detects.

catalog and are available on a made-to-order basis. Our graphical map viewer shows each assay's location on the gene, to help determine which assay is most appropriate.

Choice of Delivery Formats

Applied Biosystems delivers the assays in either a tube-format or TaqMan® Low Density Array-format (using inventoried assays only). The TaqMan® Low Density Array is a 384-well micro fluidic card that streamlines reaction set-up time, eliminates the need for liquid handling robotics, and provides standardization across multiple users and/or multiple labs. This format is ideal for analyzing many samples across a fixed number of targets, such as for biomarker screening. The TaqMan® Array arrives ready to use, with your selected TaqMan® Gene Expression Assays preloaded into each of the 384 reaction wells. Simply add 100 μ L sample mix (sample cDNA and TaqMan® Universal PCR Master Mix)

to each of the eight sample ports and run on an Applied Biosystems 7900HT Fast Real-Time PCR System. For more information, see the "TaqMan® Low Density Array" section on page 7.

A TaqMan® Genomic Assay Catalog CD is also available. This off-line version of our catalog allows you to search and select our assays without an Internet connection. Download a version today at www.allgenes.com or request a copy from your sales representative.

Convenient Assay Ordering Options

Both our online catalog and CD catalog enable searching for your target of interest by:

- Gene name
- Accession number (NCBI RefSeq ID number, etc.)
- Gene family, or functional categories and groups (kinase, transcription factor, ion channel, cytokine, etc.)

Custom TaqMan® Gene Expression Assays

- Any species or organism
- Target sequence of your choice
- Convenient single-tube format

Custom TaqMan® Gene Expression Assays are available for any species, any splice variant, or any novel gene. Simply download our free File Builder Software to format and submit your target sequence. File Builder Software can be downloaded from our Web site (<http://home.appliedbiosystems.com/support/software/filebuilder>). The software easily guides you through the ordering process from selecting the assay size, formatting your target sequence to identify the location of the probe, and submitting your order via e-mail.

File submissions are done in a secure format. Your target sequences and the associated assays that are designed are kept confidential. With Custom TaqMan® Gene Expression Assays, you benefit from Applied Biosystems proprietary software algorithms for probe and primer design, which enable you to obtain optimal assays for each target sequence. Assays are delivered in a single-tube, ready-to-use format, along with the probe and primer sequences designed from your submitted sequence.

Automation-Compatible to Accelerate High-Throughput Applications

Both TaqMan® Gene Expression Assays and Custom TaqMan® Gene Expression Assays come pre-formulated in a single, 2D-barcoded tube with an easy-to-read label. The single-tube format requires fewer set-up and pipetting steps to assemble reactions, allowing you to easily scale your throughput. Assay tubes are shipped in a 1D-barcoded



Custom TaqMan® Gene Expression Assays are delivered ready-to-use, along with the probe and primer sequences you designed.

96-position rack designed to accommodate standard liquid handling robotics and fit seamlessly into automated, high-throughput laboratory processes. Each order of assays also includes a compact disc with an assay information file that includes the assay ID numbers, detector names, reporter dye, and quencher information for easy uploading into a LIMS or sequence detection system software.

A Simple, Standardized Solution for Quantitative Gene Expression

TaqMan® Gene Expression Assays and Custom TaqMan® Gene Expression Assays are built on our 5' nuclease chemistry and consist of a FAM™ dye-labeled TaqMan® MGB probe (250 nM, final concentration), and two unlabeled PCR primers (900 nM each, final concentration). All components are QC tested and formulated

into a single 20X mix. Designed to run under universal conditions for two-step RT-PCR, TaqMan® Gene Expression Assays are simple to use. Just add TaqMan® Universal PCR Master Mix (with or without AmpErase® UNG) and your cDNA sample to generate sensitive, reproducible, and truly quantitative gene expression data on any Applied Biosystems Real-Time PCR System.

Compared to do-it-yourself methods, TaqMan® Gene Expression Assays and Custom TaqMan® Gene Expression Assays eliminate weeks or even months of probe and primer design, formulation, and testing.

For more information on this product, visit www.allgenes.com or check with your local Applied Biosystems representative.



Each endogenous control is built on our 5' nuclease chemistry and is available in a number of reporter dye/quencher formats.

TaqMan® Endogenous Controls

- Optimized, pre-formulated, ready-to-use control assays
- Cost-effective gene expression quantitation in human, mouse, rat, Arabidopsis, Drosophila, and eukaryotes
- Choice of FAM™ or VIC® dye labels

Applied Biosystems TaqMan® Endogenous Controls are a collection of pre-designed probe and primer sets that can be used to normalize the amount of sample RNA or DNA added to a reaction. For the quantitation of gene expression, deciding upon a specific control can be difficult, even when detailed information about the biological system is available. This can result in trial and error to identify an appropriate control, leading to project delays and increased costs. Applied Biosystems offers endogenous controls for the most commonly used control genes in human, mouse, rat, Arabidopsis, Drosophila, and any eukaryotic species. The assays are designed to let researchers quickly and easily identify and run the best possible endogenous control for their gene expression study.

A Simple, Standardized Solution for Quantitative Gene Expression

Each endogenous control is built on our 5' nuclease chemistry and is offered in a choice of two different reporter dyes and two quenchers:

- A FAM™ dye-labeled TaqMan® MGB probe (250 nM, final concentration) and two unlabeled PCR primers (900 nM each)
- A VIC® dye-labeled TaqMan® MGB probe (250 nM, final concentration) and two unlabeled PCR primers (150 nM each—primer limited)
- A VIC® dye-labeled TAMRA™ dye-labeled probe (250 nM, final concentration) and two unlabeled PCR primers (150 nM each—primer limited)

All components are QC tested, formulated into a single 20X mix, and functionally tested. Designed to run under universal conditions for two-step RT-PCR, our TaqMan® Endogenous Controls are simple to use. Just add TaqMan® Universal PCR Master Mix (with or without AmpErase® UNG) and your cDNA sample to generate sensitive, reproducible, and truly quantitative gene expression data on ABI PRISM® 7000 and 7700 Sequence Detection Systems, Applied Biosystems 7300 and 7500 Real-Time PCR Systems, and Applied Biosystems 7500 and 7900HT Fast Real-Time PCR Systems. Compared to do-it-yourself methods, our TaqMan® Endogenous Controls deliver a complete quantitation solution and eliminate weeks or even months of assay design, formulation, and testing.

Choosing the Right Endogenous Control

Endogenous controls can normalize the expression levels of target genes by correcting differences in the amount of cDNA that is loaded into PCR reaction wells. For best results, verify that the endogenous control is consistently expressed in the sample set to be tested. Endogenous control expression must be uniform across all samples in the study. For multiplexing, ensure that the gene expression level of the endogenous control is greater than that of the target.

Multiplex vs. Singleplex PCR

All TaqMan® Endogenous Controls that contain probes labeled with the VIC® reporter dye are primer limited. This allows multiplexing of TaqMan® Endogenous Controls with target gene expression assays, provided that the control gene is more abundantly expressed than the target gene. All TaqMan® Endogenous Controls that contain probes labeled with the FAM™ reporter dye are not primer limited and are not intended for multiplexing.

Complementary Products

TaqMan® Endogenous Controls are intended to be used with:

- TaqMan® Gene Expression Assays
- Custom TaqMan® Gene Expression Assays
- Custom TaqMan® Probes and Primers

Online Ordering

Order from our line of TaqMan® Endogenous Controls through the Applied Biosystems store at <http://store.appliedbiosystems.com>

For more information on this product, check with your local representative or visit www.allgenes.com

TaqMan® Endogenous Controls

	Dye/Quencher	Part Number	Primer Limited	Conc.	Number of Reactions, 20 µL
Eukaryotic 18S rRNA	VIC®/MGB	4319413E	Y	20X	2,500
	VIC®/TAMRA™	4310893E	Y	20X	2,500
	FAM™/MGB	4333760T	N	20X	125
	FAM™/MGB	4333760f	N	20X	500
Human ACTB (beta actin)	VIC®/MGB	4326315E	Y	20X	2,500
	VIC®/TAMRA™	4310881E	Y	20X	2,500
	FAM™/MGB	4333762T	N	20X	125
	FAM™/MGB	4333762F	N	20X	500
Human B2M (beta-2-microglobulin)	VIC®/MGB	4326319E	Y	20X	2,500
	VIC®/TAMRA™	4310886E	Y	20X	2,500
	FAM™/MGB	4333766T	N	20X	125
	FAM™/MGB	4333766F	N	20X	500
Human GAPD (GAPDH)	VIC®/MGB	4326317E	Y	20X	2,500
	VIC®/TAMRA™	4310884E	Y	20X	2,500
	FAM™/MGB	4333764T	N	20X	125
	FAM™/MGB	4333764F	N	20X	500
Human GUSB (beta glucuronidase)	VIC®/MGB	4326320E	Y	20X	2,500
	VIC®/TAMRA™	4310888E	Y	20X	2,500
	FAM™/MGB	4333767T	N	20X	125
	FAM™/MGB	4333767F	N	20X	500
Human HPRT1	VIC®/MGB	4326321E	Y	20X	2,500
	VIC®/TAMRA™	4310890E	Y	20X	2,500
	FAM™/MGB	4333768T	N	20X	125
	FAM™/MGB	4333768F	N	20X	500
Human PGK1 (phosphoglyceratekinase 1)	VIC®/MGB	4326318E	Y	20X	2,500
	VIC®/TAMRA™	4310885E	Y	20X	2,500
	FAM™/MGB	4333765T	N	20X	125
	FAM™/MGB	4333765F	N	20X	500
Human PPIA (cyclophilin A)	VIC®/MGB	4326316E	Y	20X	2,500
	VIC®/TAMRA™	4310883E	Y	20X	2,500
	FAM™/MGB	4333763T	N	20X	125
	FAM™/MGB	4333763F	N	20X	500
Human RPL0 (large ribosomal protein)	VIC®/MGB	4326314E	Y	20X	2,500
	VIC®/TAMRA™	4310879E	Y	20X	2,500
	FAM™/MGB	4333761T	N	20X	125
	FAM™/MGB	4333761F	N	20X	500
Human TBP (TATA-box binding protein)	VIC®/MGB	4326322E	Y	20X	2,500
	VIC®/TAMRA™	4310891E	Y	20X	2,500
	FAM™/MGB	4333769T	N	20X	125
	FAM™/MGB	4333769F	N	20X	500
Human TFRC (CD71) (transferring receptor)	VIC®/MGB	4326323E	Y	20X	2,500
	VIC®/TAMRA™	4310892E	Y	20X	2,500
	FAM™/MGB	4333770T	N	20X	125
	FAM™/MGB	4333770F	N	20X	500
Mouse GAPD (GAPDH)	VIC®/MGB	4352339E	Y	20X	2,500
Mouse ACTB (beta actin)	VIC®/MGB	4352341E	Y	20X	2,500
Rat GAPD (GAPDH)	VIC®/MGB	4352338E	Y	20X	2,500
Rat ACTB (beta actin)	VIC®/MGB	4352340E	Y	20X	2,500

Table 1.

TaqMan® Low Density Array

- TaqMan® performance in a low density array format
- Micro fluidic technology provides access to 384-well format without liquid-handling robotics
- Standardization enables screening of multiple samples against selected gene panels

The TaqMan® Low Density Array is an easy-to-use micro fluidic card for real-time PCR. The micro fluidic technology utilizes eight sample-loading ports, each connected to 48 reaction chambers, streamlining the reaction set-up process and eliminating the need for liquid-handling robotics. Simply load 100 µL of sample mix, (30–100 ng of cDNA sample and 50 µL of 2X TaqMan® Universal PCR Master Mix), into each of the sample loading ports. Centrifuge briefly, seal the TaqMan® Array, and run on the Applied Biosystems 7900HT Fast Real-Time PCR System.

TaqMan® Low Density Arrays are delivered with your choice of inventoried TaqMan® Gene Expression Assays pre-loaded into each of the 384 reaction wells. Select from our collection of more than 40,000 inventoried

assays to run on the TaqMan® Array (for selection, visit www.allgenes.com). TaqMan® Arrays are available in nine different formats, covering 12, 16, 24, 32, 48, 64, 96 (2 choices), and 384 assays per array (Table 3). TaqMan® Arrays are designed for use on the flexible Applied Biosystems 7900HT Fast Real-Time PCR System.

Ideal Screening Technology

TaqMan® Low Density Arrays are ideal for screening applications such as:

- Biomarker screens
- Toxicology screens
- Target class screens
- Pathway analysis
- Disease set analysis

In addition to Custom TaqMan Arrays, Applied Biosystems also offers two versions of TaqMan® Low Density Array Panels—the TaqMan® Low Density Array Immune Panel and the TaqMan® Low Density Array Endogenous Control Panel. The immune panel contains 96 TaqMan Gene Expression Assays pre-loaded in quadruplicate across the 384 reaction wells. The assays were selected in collaboration with the Immune Tolerance Network, as well as other

researchers in the field. All assays are designed to genes with known implications in immune response. For convenience, these TaqMan® Array Immune Panels are available in packs of ten.

The endogenous control panel allows researchers to quickly identify the optimal gene(s) to use as endogenous controls for each gene expression study. Eleven TaqMan® Endogenous Controls for human, plus five additional TaqMan Gene Expression Assays (identified as exhibiting minimal variation in gene expression across sixteen human tissues), are pre-loaded in the TaqMan® Array in triplicate. Eight samples can be run on each array. The TaqMan® Array Endogenous Control Panels are available in packs of two.

Applied Biosystems 7900HT Fast Real-Time PCR System

The TaqMan Array was designed expressly for the Applied Biosystems 7900HT Fast Real-Time PCR System, a flexible, high-throughput, Real-Time PCR system that detects and quantitates nucleic acid sequences. The combination of the TaqMan Array and the 7900HT system helps make high-throughput gene expression research successful and easy.

TaqMan® Low Density Array

Description	# of Assays	# of Samples per Card				Minimum Order Quantities	Part Number
		1 Replicate	2 Replicates	3 Replicates	4 Replicates		
Format 12	11 + 1 control				8	20	4342247
Format 16	15 + 1 control			8		20	4346798
Format 24	23 + 1 control		8		4	20	4342249
Format 32	31 + 1 control			4		20	4346799
Format 48	47 + 1 control		4		2	20	4342253
Format 64	63 + 1 control			2		20	4346800
Format 96a	95 + 1 control		2		1	20	4342259
Format 96b	95 + 1 control		2		1	20	4342261
Format 384	380 + 1 control	1				50	4342265
Immune Panel	96		2		1	10	4342510
Endogenous Control Panel	16			8		2	4367563
7900HT TaqMan® Low Density Array Upgrade*							4329012

Includes: sample block, micro fluidic card sealer, custom buckets and adaptors, *Getting Started* guide, and chemical installation kit.

*A compatible centrifuge is required but not supplied.

Table 2.

Custom TaqMan® Probes and Primers

- Choice of dye labels, quenchers, and synthesis scales
- Available for any species or organism
- For use in quantitative gene expression, SNP genotyping, other allelic discrimination applications, and pathogen detection

When you know the exact sequences you need for your TaqMan® Probes and Primers, Applied Biosystems can synthesize them for you. As the market leader in real-time PCR, our high-quality custom products can be used in all of your real-time and end-point PCR applications. These products offer you the ideal in flexibility, whether you prefer to optimize your own reaction formulation, or if you simply want to buy in large quantities.

Our Custom TaqMan® Probes and Primers are manufactured at three sites around the world—the United States, the United Kingdom, and Japan, to offer excellent delivery time. Order by fax, e-mail, or online, and send your sequences to our synthesizers electronically, speeding order delivery time.

Choice of Quenchers

Applied Biosystems Custom TaqMan® Probes incorporate a 5' reporter dye and a 3' quencher. Our most popular quencher is a non-fluorescent quencher (NFQ) combined with an MGB (minor groove binder) moiety. The NFQ offers the advantage of lower background signal, which results in better precision in quantitation. The MGB moiety stabilizes the hybridized probe and effectively raises the melting temperature (T_m). This means that MGB probes can be shorter than traditional dual-labeled probes, which make them better suited for allelic discrimination applications. The shorter probe lengths mean that single base mismatches (e.g., SNPs)

will have a greater destabilizing effect on an MGB probe, resulting in better discrimination. The shorter length also offers greater design flexibility for all real-time PCR applications.

Applied Biosystems offers the traditional dual-labeled Custom TaqMan® Probes with a TAMRA™ dye fluorescent quencher as well. All TAMRA™ dye TaqMan® probes are HPLC purified.

A Selection of Reporter Dyes

Applied Biosystems Custom TaqMan® Probes can be ordered with a variety of different reporter dyes to facilitate your multiplexing applications.

Synthesis Scales

TaqMan® Custom Probes and Primers are available in a choice of three standard sizes. Each includes a pre-defined quantity of probe or primer to ensure that you get the same amount each time you order and aren't subject to variations in synthesis yield. For larger synthesis scales on these products, please contact your Applied Biosystems Sales Representative.

Primer Express® Design Software

Applied Biosystems Primer Express® software is available to simplify the probe and primer design process. Primer Express® is available for individual users and in multi-user packs. Please check our Web site at www.appliedbiosystems.com for more details.

And, if you prefer the convenience of a pre-designed probe and primer set, check our vast online selection of TaqMan® Gene Expression and SNP Genotyping Assays.

Other Fluorescent Dye-Labeled Oligos

Applied Biosystems also offers a host of other custom oligo products for use in many applications, including microsatellite-based linkage mapping, mutation detection, and more. Please see our Web site, www.appliedbiosystems.com for more details.

Probe Type (3' Quencher)	5' Reporter Dyes	Probe Lengths
TaqMan® Custom MGB Probes	FAM™, VIC®, TET™, or NED™*	13-25 bases
TaqMan® Custom TAMRA™ Dye Probes	FAM™, VIC®, or TET™	Up to 35 bases

Table 4.

*Please note that NED™ dye can give lower signal intensity than FAM™, VIC®, or TET™ dye on most Real-Time PCR systems. The Applied Biosystems 7500 Real-Time PCR System has been optimized to yield higher signal intensity for NED™ dye.

Custom TaqMan® Probes and Primers	Part Number	Reporter Dye Label	Quantity
TaqMan® TAMRA™ Probes	450025	6-FAM™, VIC® or TET™	6,000 pmol
	450024	6-FAM™, VIC® or TET™	20,000 pmol
	450003	6-FAM™, VIC® or TET™	50,000 pmol
TaqMan® MGB Probes	4316034	6-FAM™, VIC®, NED™ or TET™	6,000 pmol
	4316033	6-FAM™, VIC®, NED™ or TET™	20,000 pmol
	4316032	6-FAM™, VIC®, NED™ or TET™	50,000 pmol
Real-Time PCR Primers (sequence detection primers)	4304970	No label	10,000 pmol
	4304971	No label	80,000 pmol
	4304972	No label	130,000 pmol

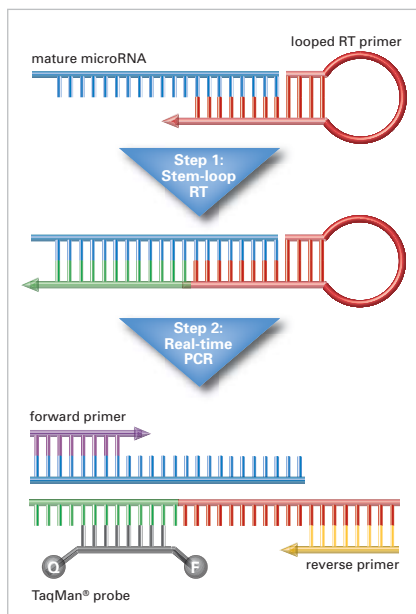
Table 5.

TaqMan® MicroRNA Assays

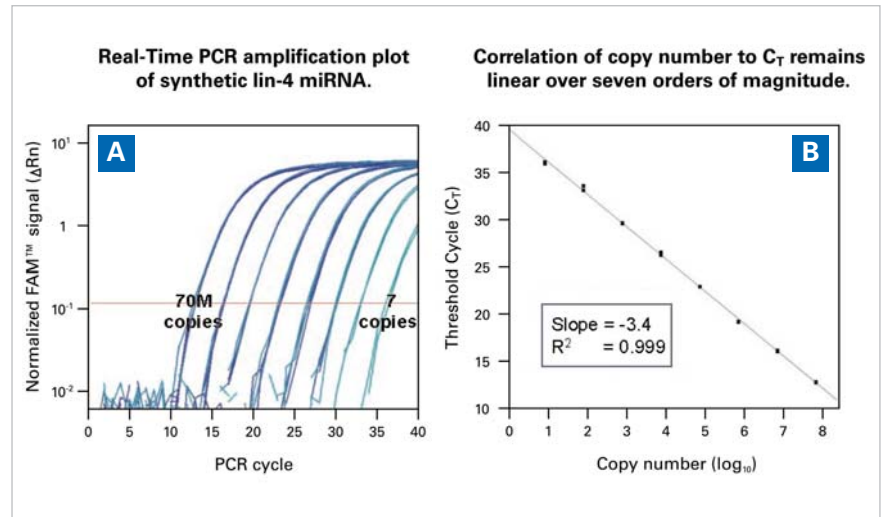
- Highly specific—quantitate only mature miRNAs, not inactive precursors
- Fast, simple, and scalable—two-step assay provides high-quality results in less than three hours
- Sensitive—conserves limited samples by requiring only 1–10 *nanograms* of total RNA or equivalent
- Wide dynamic range—up to seven logs

By making novel adaptations in assay design, Applied Biosystems is now able to bring the specificity, sensitivity, and simplicity of TaqMan® assays and Real-Time PCR to miRNA detection and quantitation.

The basis of TaqMan® MicroRNA Assays is a target-specific stem-loop, reverse-transcriptase primer. Its innovative design (below) overcomes a fundamental problem in miRNA quantitation: the short length of mature miRNAs (~22 nt) prohibits



TaqMan® MicroRNA Assay mechanism. A simple, two-step mechanism brings the advantages of Real-Time PCR to miRNA quantitation.



Unparalleled dynamic range. Amplification plot of synthetic miRNA lin-4 by stem-loop RT-PCR. Estimated synthetic miRNA input is based on OD: 70, 700, 7,000, 70,000, 700,000, 7M, and 70M copies in PCR. Significant correlation between the copy number and C_T value up to seven orders of magnitude. A) amplification plot, B) amplification plot converted to log scale.

conventional design of a random-primed RT step followed by a specific real-time assay. The stem-loop accomplishes two goals: 1) specificity for only the mature miRNA target, and 2) formation of a RT primer/mature miRNA-chimera, extending the 5' end of the miRNA. The resulting longer RT amplicon presents a template amenable to Real-Time TaqMan assay design with great specificity.

To ensure accurate results, every individual TaqMan MicroRNA Assay design has been functionally validated under laboratory conditions.

Distinguish Between Highly Homologous Mature miRNAs

TaqMan® miRNA assays are not only specific for mature miRNAs, they can also successfully distinguish between highly homologous targets, and in some cases, with only a single nucleotide mismatch. This means that researchers can be confident that they are measuring mature, biologically relevant miRNAs, not precursors, and that closely related miRNA variants have been targeted with very high specificity.

Requires Only Minimal Starting Material

TaqMan miRNA assays are extremely sensitive—researchers need only 1–10 *nanograms* of purified total RNA or equivalent to reliably quantify their miRNAs of interest, not the several *micrograms* typically required for hybridization-based methods. Such sensitivity enables researchers to conduct more extensive experiments with limited starting material, as with full-panel miRNA expression profiling. It also conserves researcher's precious or difficult-to-obtain materials, such as stem cells or matched tumor/normal human cancer samples.

Unparalleled Dynamic Range

TaqMan miRNA assays can deliver up to seven logs of linear dynamic range, from a few copies, to millions of copies (data above). This means that researchers will get accurate data whether their materials contain few or many molecules of the target miRNA per cell—an important factor given the wide range of miRNA concentrations across different cells, tissue types, and disease states.

This increased accuracy also makes TaqMan miRNA assays highly reproducible among replicates and when performed by different researchers.

Fast Time-to-Results

By taking advantage of gold-standard TaqMan® reagent-based technology with universal thermal cycling conditions, TaqMan miRNA assays are familiar, fast, and easy to set up. Just start with your total RNA sample, and get results in less than three hours using any Applied Biosystems Real-Time PCR System.

A Convenient and Scalable Solution

TaqMan miRNA assays are pre-designed, functionally validated, and available off-the-shelf from Applied Biosystems, making them extremely convenient. Spend your valuable time generating results, not designing and troubleshooting assays.

The TaqMan miRNA assay format is easily amenable to high-throughput screening methods. All of the up to 157 individual miRNA assays and controls contained in the Human Panel – Early Access Kit fit into two 96-well plates. For example, a statistically rigorous experiment profiling the entire assay panel in quadruplicate, including multiple control assays, can be run in just two 384-well plates.

The Human Panel Kit provides flexibility—researchers can run the entire panel together, or any subset of assays they wish. This scalable solution accommodates both large initial miRNA panel screens, and further in-depth experiments.

Cost-Effective

With a cost-per-target comparable to microarray experiments, TaqMan MicroRNA Assays are a powerful alternative. Not only are they convenient and adaptable to high-throughput protocols, they also offer the superior specificity, sensitivity, and dynamic range of Real-Time PCR.

Case Study: Comparison to Northern Analysis

Recent studies showed that northern analysis and TaqMan MicroRNA Assay results correlated when both methods were performed under ideal conditions. However, TaqMan miRNA assays are a better choice because:

- They can offer a high level of discrimination between highly homologous miRNA variants
- They are easy to use and provide results within three hours
- They are highly sensitive, requiring minimal starting materials
- They have a dynamic range of up to seven logs

Early Access Product Availability

Applied Biosystems has released the following two early access miRNA products:

1. TaqMan® MicroRNA Assays Human Panel – Early Access Kit

This product includes 157 individual miRNA assays and three non-human negative control assays. The panel covers the majority of human miRNAs currently identified in the Sanger miRNA Registry (<http://microrna.sanger.ac.uk>).

The kit supplies sufficient materials to perform 20 reactions (20 µL volume) of each assay—enough for several full-panel miRNA screens.

Because many mature miRNA sequences are identical across closely related species, the majority of assays in the Human Panel Kit are also valid for mouse and rat targets. A cross-species chart detailing the human, mouse, and rat microRNAs targeted by each individual assay can be downloaded from the Web at mirna.appliedbiosystems.com

2. TaqMan® MicroRNA Assay ABM 000008 hsa-miR-16

This is an individually available assay targeting the mature human miRNA miR-16. The assay allows customers to easily evaluate TaqMan MicroRNA Assay technology without having to invest in the full human panel kit. Identified as a candidate positive control for human miRNA quantification experiments, miR-16 exhibits a high level of expression across most tissue types. Also, because the mature miR-16 sequence is identical between human, mouse, and rat, it is also useful for quantifying the mouse and rat miR-16 miRNAs.

Additional TaqMan MicroRNA Assay products will become available in the near future. Register for product updates on the Web at mirna.appliedbiosystems.com



Applied Biosystems 7900HT Fast Real-Time PCR System

Applied Biosystems Real-Time PCR Systems

Applied Biosystems Real-Time PCR Systems make real-time PCR more accessible than ever before by providing powerful solutions to fit the needs of any laboratory. These systems are easy to use with next generation software, and of course, they're backed by Applied Biosystems unmatched track record of performance, quality, and long-term reliability.

Applied Biosystems 7900HT Fast Real-Time PCR System—the ultimate in performance and flexibility

- User-interchangeable block options include 384-well, 96-well, TaqMan® Low Density Array, and Fast 96-well (for results in about 35 minutes)
- Extended-life 488 nm argon-ion laser combined with continuous wavelength detection from

500–660 nm, provides unmatched dye resolution capabilities

- Automation Accessory provides walk away automation for unmatched throughput
- Enterprise Edition Software enables hundreds to thousands of plates to be analyzed simultaneously and assists with 21CFR part 11 compliance

Applied Biosystems 7500 Fast and 7500 Real-Time PCR Systems—versatile platforms for users requiring extended capabilities

- Advanced five color optical configuration supports a broader range of fluorophores, with variable excitation capability allowing greater sensitivity for longer wavelength (red) dyes
- A new high-speed 96-well thermal cycling block enables real-time PCR results in under 40 minutes



Applied Biosystems 7500HT Fast Real-Time PCR System

- Relative Quantitation Software allows gene expression data from up to ten plates to be combined in one analysis

Applied Biosystems 7300 Real-Time PCR System—an economical solution setting the standard for the basic researcher

- Four color detection provides the flexibility to perform all major applications
- Powerful and versatile software makes experimental set-up and data processing simple and straightforward
- Precision optics and a charge-coupled device (CCD) camera provide highly accurate, reproducible, and reliable results
- Patented sample temperature control provides superior reproducibility and consistent, high-quality results

Product Specification Comparison

	Part Number	Fill Volumes	Number of 20 μ L Reactions	Available Reporter Dye Labels	Universal Formulation	Approximate Delivery Time
TaqMan[®] Gene Expression Assays						
Inventoried	4331182	250 μ L, 20X	250	FAM [™]	Yes	2 – 3 days
Non-Inventoried	4351372	360 μ L, 20X	360	FAM [™]	Yes	10 – 14 days
Custom TaqMan[®] Gene Expression Assays	4331348	360 μ L, 20X	360	FAM [™]	Yes	10 – 14 days
	4332078	750 μ L, 20X	750	FAM [™]	Yes	10 – 14 days
	4332079	967 μ L, 60X	2,900	FAM [™]	Yes	10 – 14 days
TaqMan[®] Endogenous Controls						
Primer limited	Various — see Table 1			VIC [®]	Yes	
Not primer limited	Various — see Table 1			FAM [™]	Yes	
Custom TaqMan[®] Probes	Various — see Table 5			FAM [™]	No	4 – 7 days
	Various — see Table 5			VIC [®]	No	4 – 7 days
	Various — see Table 5			TET [™]	No	4 – 7 days
	Various — see Table 5			NED [™]	No	4 – 7 days
	Part Number	Fill Volumes (each assay)	Number of Reactions	Available Reporter Dye Labels	Universal Formulation	Approximate Delivery Time
TaqMan[®] MicroRNA Assays (Early Access Products)						
Human Panel Kit (Includes 157 human miRNA assays, and additional non-human control assays, for a total of up to 3,680 reactions)	4365409	RT: 60 μ L, 5X; 15 μ L rxn vol. TaqMan [®] Assay: 40 μ L, 10X; 20 μ L rxn vol.	Up to 3,680 • 157 individual miRNA assays (20 rxn each) • Additional negative control assays (20 rxn each)	FAM [™]	Yes	2 – 3 days
Individual hsa-miR-16 assay (Targets human, mouse, and rat miR-16)	4365746	RT: 450 μ L, 5X 15 μ L rxn vol. TaqMan [®] Assay: 300 μ L, 10X; 20 μ L rxn vol.	150	FAM [™]	Yes	2 – 3 days
	Part Number	Reaction Size	Number of 1 μ L Reactions	Available Assays	Approximate Delivery Time	
Custom TaqMan[®] Low Density Arrays	Various — see Table 2		384	Any Inventoried TaqMan Gene Expression Assay	6 – 8 weeks	
TaqMan[®] Low Density Array Panels	Various — see Table 2		384	Defined Panels of TaqMan Gene Expression Assays	2 – 3 days	

Table 6.

For Research Use Only. Not for use in diagnostic procedures.

NOTICE TO PURCHASER:

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