

TaqMan® MicroRNA Assays

Quantitate microRNAs with the specificity and sensitivity of TaqMan® assay chemistry

- Highly specific—quantitate only the biologically active mature miRNAs
- Sensitive—conserves limited samples; requires only 1–10 *nanograms* of total RNA or equivalent
- Wide dynamic range—up to seven logs—detect high and low expressors in a single experiment
- Fast, simple and scalable—two-step qRT-PCR assay provides high-quality results in less than three hours



MicroRNAs (miRNAs) are a class of naturally occurring non-coding RNAs that play a role in gene regulation. These transcripts are highly conserved, single-stranded RNAs (~22 nucleotides) that are cleaved from larger hairpin precursor transcripts (Figure 1). MicroRNAs use the RNA interference pathway to affect gene regulation by either cleaving, or most often, repressing the translation of their messenger RNA (mRNA) targets.

TaqMan® MicroRNA Assays— A Real-Time PCR Revolution

By making novel adaptations in assay design, Applied Biosystems is able to bring our gold standard specificity, sensitivity and reproducibility of TaqMan® assays and quantitative real-time PCR to miRNA detection and quantitation.

The basis of TaqMan® MicroRNA Assays is a target-specific stem-loop structure, reverse-transcription primer (Figure 2). Its innovative design overcomes a fundamental problem in miRNA quantitation: the short length of mature miRNAs

(~22 nt) prohibits 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 standard real-time PCR, using TaqMan assays.

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

Distinguish Between Highly Homologous Mature miRNAs

TaqMan MicroRNA Assays are not only specific for mature miRNAs, they also distinguish between highly homologous targets. As many miRNA family members (i.e. the let-7 miRNA family) differ in sequence by as little as one base, real-time PCR using TaqMan assays, gives the specificity needed for differentiation (Figure 3).

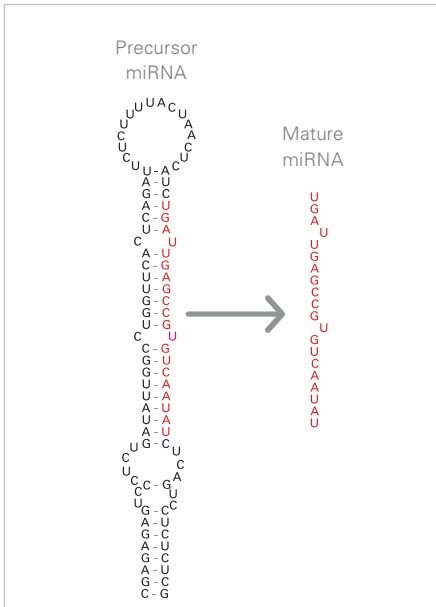


Figure 1. Mechanism for producing mature microRNAs. Active mature miRNAs are cleaved from longer inactive precursors. The inherent homology between the active and precursor molecules can add complexity to miRNA analysis.

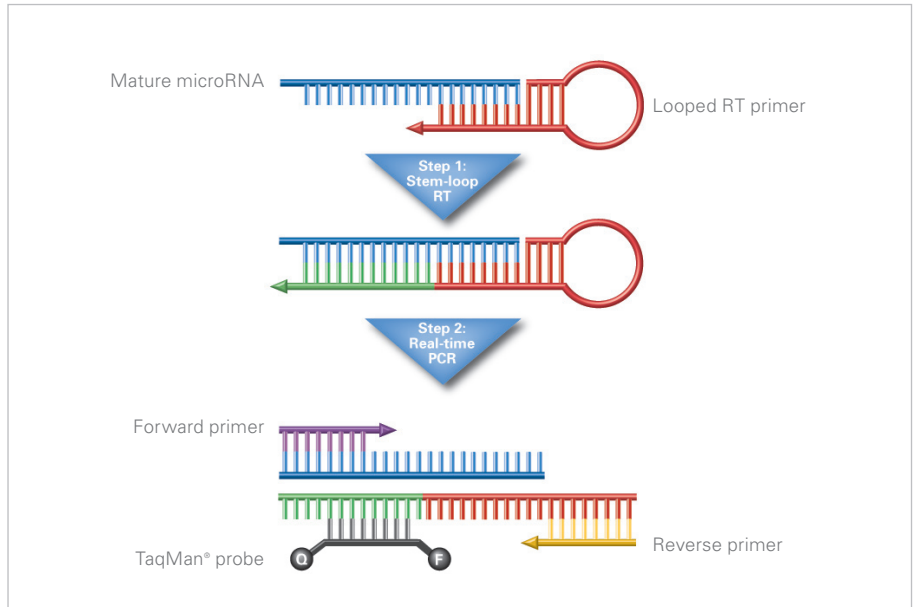


Figure 2. TaqMan® MicroRNA Assay mechanism. A simple, two-step mechanism brings the advantages of real-time PCR to miRNA quantitation.

Requires Only Minimal Starting Material

TaqMan MicroRNA 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.

Unparalleled Dynamic Range

TaqMan MicroRNA Assays deliver the wide linear dynamic range TaqMan assays are known for—up to *seven logs*. This means that researchers can accurately quantitate miRNA targets varying from a few copies to millions of copies in the same experiment—an important factor given the wide range of miRNA concentrations within and across different cells, tissue types and disease states.

Fast Time-to-Results

By taking advantage of gold-standard TaqMan® reagent-based technology with universal thermal cycling conditions, TaqMan MicroRNA 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.

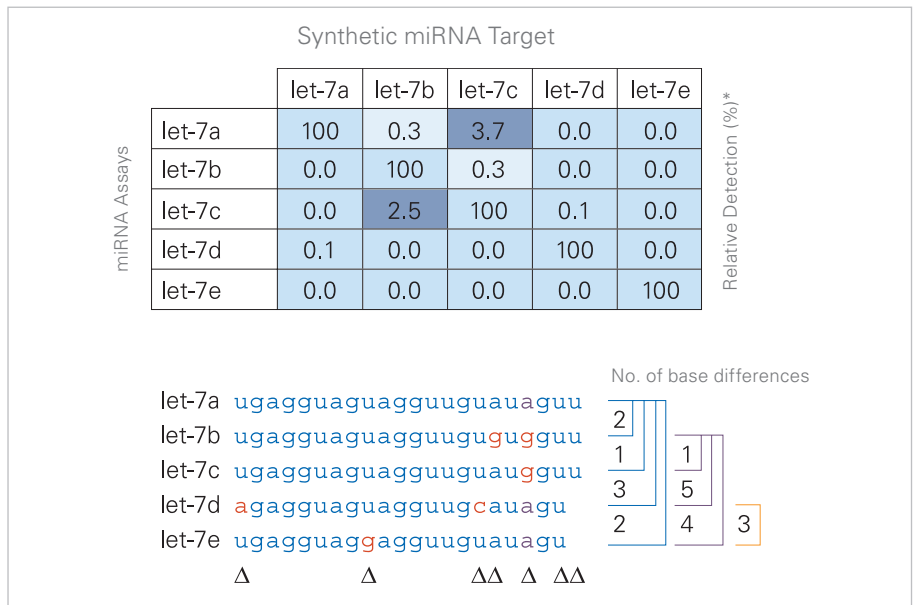


Figure 3. Single-base discrimination of TaqMan® MicroRNA Assays. Relative detection (%) is calculated based on C_T difference between perfectly matched and mismatched assays.

Convenient and Scalable Solution

TaqMan MicroRNA 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.

TaqMan® MicroRNA Assays

TaqMan MicroRNA Assays are available for a range of species, including human, mouse, rat, *Drosophila*, *C. elegans* and *Arabidopsis*. Endogenous controls for human and mouse assays are also available. Applied Biosystems will continue to increase the number of

TaqMan MicroRNA Assays for these species, with the goal of keeping aligned with the Sanger miRBase Registry (<http://microRNA.sanger.ac.uk/sequences/index.shtml>).

TaqMan® MicroRNA Reverse Transcription (RT) Kit

The TaqMan® MicroRNA RT Kit provides the necessary components for optimal performance of TaqMan MicroRNA Assays. Components of this kit are used with the RT primer provided with the MicroRNA Assay to convert miRNA to cDNA. This kit is available in 200 or 1,000-reaction sizes.

The TaqMan® Assay Advantage

TaqMan MicroRNA Assays address the challenges in miRNA quantitation by providing:

- **High specificity**, including discrimination of mature miRNAs from inactive precursors
- **High sensitivity**, providing reliable results from just *nanograms* of starting material
- **Accuracy over a wide dynamic range**, providing confidence that miRNAs will be detected regardless of copy number
- **Speed**, providing scalable results in under three hours

Applied Biosystems and Ambion—A Perfect Match

Applied Biosystems recently acquired the Research Products Division of Ambion. With the acquisition and eventual full integration of Ambion into Applied Biosystems, we will be able to provide you with the most

comprehensive portfolio of innovative products and solutions for your miRNA research needs.

The *mirVana*™ miRNA Isolation Kit enables the rapid isolation of total RNA without loss of microRNAs. Unlike other filter-based procedures that result in loss of small RNA <200 nt, the *mirVana* miRNA Isolation Kit isolates total RNA ranging in size from kilobases down to 10-mers. The rapid 30-minute procedure delivers exceptionally pure and intact total RNA from a wide range of tissue and sample types including tissues rich in nucleases. An optional procedure to further isolate small RNA <200 nt is also included.

Applied Biosystems Real-Time PCR Instrumentation

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 384-well or Fast 96-well (for results in 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 21 CFR 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 high-speed 96-well thermal cycling block option enables real-time PCR results in under 40 minutes
- User-customizable SDS v1.4 21CFR11 Module offers all the tools needed to comply with FDA 21CFR Part 11 regulations

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



Applied Biosystems 7900HT Fast Real-Time PCR System



Applied Biosystems 7500 Fast Real-Time PCR System

ORDERING INFORMATION

Product Description

Part Number

TaqMan® MicroRNA Assays

Multiple‡

Contents:

- RT primer tube: 5X reaction mix
- TaqMan® Assay tube: 20X reaction mix
- 150 real-time PCR reactions (20 µL)

Related Products Required for Use with TaqMan® MicroRNA Assays

TaqMan® MicroRNA RT Kit, 200 reactions	4366596
TaqMan® MicroRNA RT Kit, 1,000 reactions	4366597
TaqMan® Universal Master Mix, No AmpErase® UNG (5 mL)	4324018
TaqMan® Universal Master Mix, No AmpErase® UNG (2 x 5 mL)	4364341
TaqMan® Universal Master Mix, No AmpErase® UNG (10 x 5 mL)	4324020
<i>mirVana</i> ™ miRNA Isolation Kit (40 total RNA purifications)	Cat# 1560

‡ For the latest information on TaqMan® MicroRNA Assays, including a list of available assays, please visit mirna.appliedbiosystems.com, for information on Ambion microRNA products, visit www.ambion.com

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Headquarters

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

International Sales

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