TaqMan Advanced miRNA Assays

Key features

- **Universal reverse transcription (RT)**—one RT step for all Applied Biosystems™ TaqMan® Advanced miRNA Assays
- **Sensitive**—detect as few as 60 copies of input microRNA (miRNA)
- **Specific**—detect only mature miRNA and distinguish between highly homologous miRNAs
- **Small sample input**—detect and quantify mature miRNA from as little as 1 pg of total RNA or 2 µL of plasma or serum
- **Versatile**—compatible with tissue and biofluids including serum and plasma

MicroRNAs (miRNAs) are short (19–24 nucleotides in length), noncoding RNAs that posttranscriptionally regulate gene expression and control diverse biological processes including cell proliferation, cell fate determination, and cell death. miRNAs have significant promise as biomarkers for diseases, given their regulatory role in many cellular processes combined with their stability in samples such as plasma, serum, and tissue. Circulating miRNAs are easily accessible via serum samples, and differential expression of miRNAs in healthy versus diseased research samples may be used to detect or monitor disease progression in the future. The short length, low abundance, and sequence similarity of many biologically important miRNAs can lead to challenges in studying them. Thus, choosing the right tools is critical for a successful miRNA experiment.

Streamlined workflow with high sensitivity and specificity

TaqMan Advanced miRNA Assays and the Applied Biosystems™ TaqMan® Advanced miRNA cDNA Synthesis Kit have been specially designed to quantify mature miRNAs using real-time PCR (qPCR). Ideal for analysis of multiple miRNA targets from a single sample, the TaqMan Advanced miRNA cDNA Synthesis Kit has a universal RT step to simplify and streamline the workflow (Figure 1). After sample preparation, cDNA is synthesized by 3′ poly(A) tailing and 5′ ligation of an adaptor sequence to extend the miRNA at each end prior to RT. The cDNA is then preamplified using universal primers and a master mix to uniformly increase the amount of cDNA for each target, maintaining the relative differential levels. Unlike traditional preamplification, these primers recognize the universal sequences added to every miRNA at the 5′ and 3′ ends, helping to ensure there is no amplification bias.

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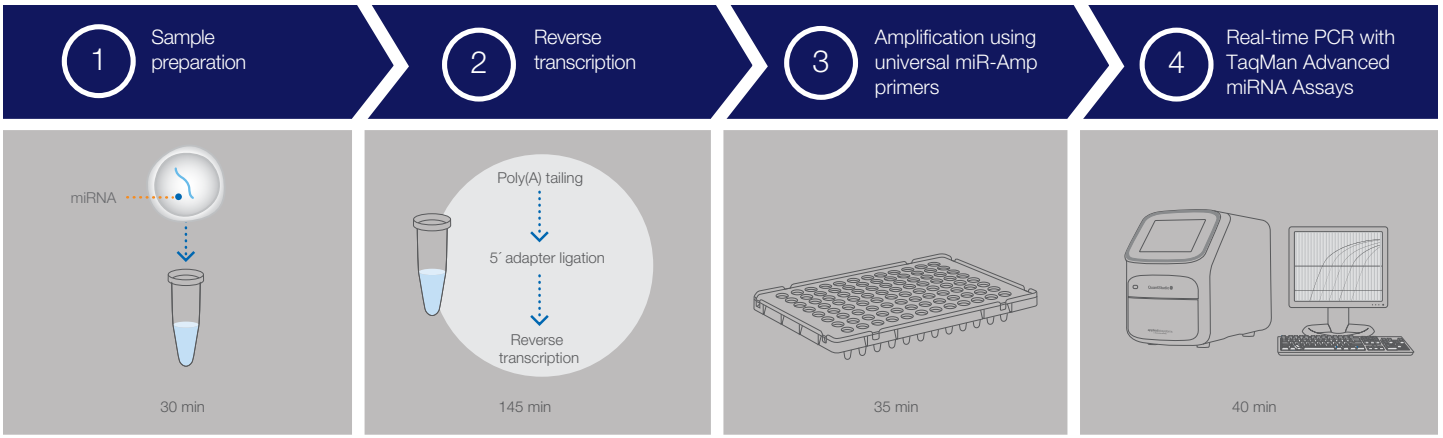

Figure 1. The TaqMan Advanced miRNA Assay workflow.

TaqMan Advanced miRNA Assays are then used to quantitate each miRNA target by qPCR. Drawing from the proprietary Applied Biosystems™ bioinformatics assay design pipeline, TaqMan Advanced miRNA Assays are preformulated primer and probe sets designed to detect and quantify a large range of mature miRNAs. Representing some of the most sensitive and specific assays available, TaqMan Advanced miRNA Assays provide up to 6 logarithmic units of dynamic range using as little as 1 pg of total RNA from tissue or 2 µL of eluant from serum or plasma. In addition, these assays exhibit high specificity with little to no cross-reactivity between closely related miRNA family members.

Superior sensitivity in tissue, serum, and plasma

In situations where sensitivity is crucial, such as when using miRNAs as biomarkers, TaqMan Advanced miRNA Assay chemistry offers a clear advantage over other commercially available kits across a range of serum or plasma samples (Figure 2). This system is compatible with the typically minute amounts of RNA in serum and plasma, to support the study of circulating miRNAs. In addition, the unique universal RT system is ideal for samples that are limited in quantity, and the cDNA generated from a single reaction can be stored frozen, ready for any number of possible uses.

Gold-standard TaqMan Assay specificity

When closely related mature miRNAs differ by as little as one base, it is important to use tools with the power to discriminate between these highly similar targets. The specificity of TaqMan Advanced miRNA Assays is demonstrated using a panel of closely related let-7 miRNAs (Figure 3). Each assay was tested individually against synthetic miRNAs for members of the let-7 family, with the C_t differences used to calculate the percent relative detection. There is minimal or no cross-reactivity between each member of the let-7 family.

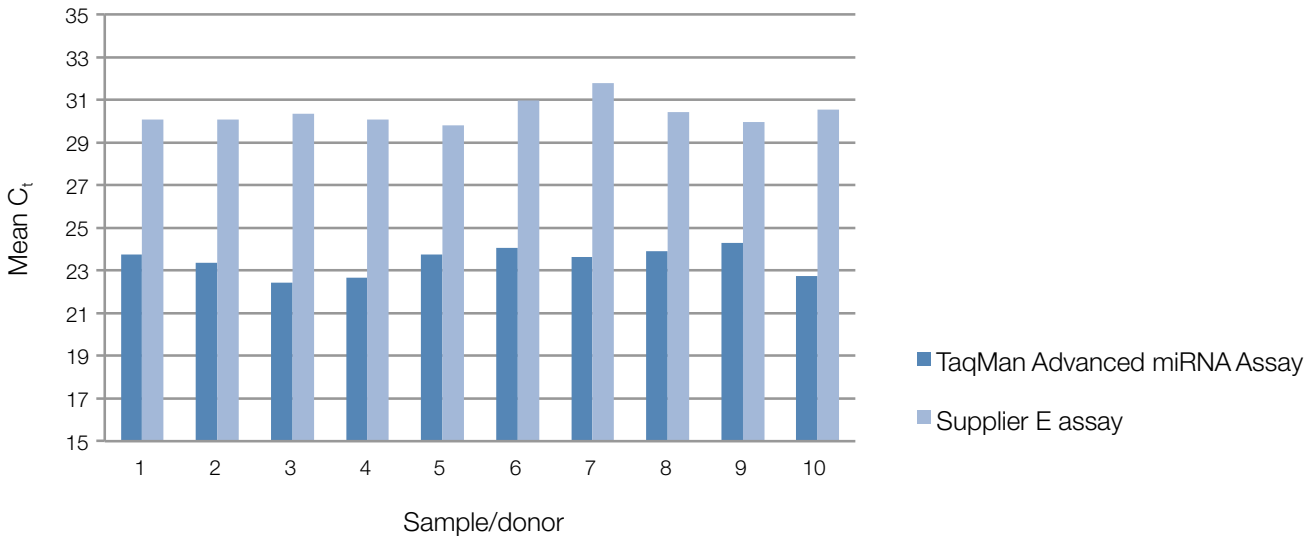

Figure 2. Sensitivity of miRNA assays using serum samples. Data for the hsa-miR-145-5p TaqMan Advanced miRNA Assay on serum samples from 10 different donors are compared with those of the corresponding assay from another supplier.

	Synthetic template							
TaqMan Advanced miRNA Assay	Let-7a	Let-7b	Let-7c	Let-7d	Let-7e	Let-7f	Let-7g	Let-7i
Let-7a	100%	0%	0%	0%	4%	2%	0%	0%
Let-7b	0%	100%	3%	0%	0%	0%	0%	0%
Let-7c	1%	2%	100%	0%	0%	0%	0%	0%
Let-7d	0%	0%	0%	100%	0%	0%	0%	0%
Let-7e	0%	0%	0%	0%	100%	0%	0%	0%
Let-7f	1%	0%	0%	0%	0%	100%	0%	0%
Let-7g	0%	0%	0%	0%	0%	0%	100%	4%
Let-7i	0%	1%	0%	0%	0%	0%	0%	100%

miRNA name	miRNA sequence
hsa-let-7a-5p	UGA GGU AGU AGG UUG UAU AGU U
hsa-let-7b-5p	UGA GGU AGU AGG UUG UGU GGU U
hsa-let-7c-5p	UGA GGU AGU AGG UUG UAU GGU U
hsa-let-7d-5p	AGA GGU AGU AGG UUG CAU AGU U
hsa-let-7e-5p	UGA GGU AGG AGG UUG UAU AGU U
hsa-let-7f-5p	UGA GGU AGU AGA UUG UAU AGU U
hsa-let-7g-5p	UGA GGU AGU AGU UUG UAC AGU U
hsa-let-7i-5p	UGA GGU AGU AGU UUG UGC UGU U
	* * * * * *

Figure 3. Assay specificity on closely related miRNAs. TaqMan Advanced miRNA Assays demonstrate little to no cross-reactivity between highly homologous members of the let-7 miRNA family. Differences in nucleotide sequences are indicated by asterisks (*).

TaqMan Advanced miRNA Assays provide high specificity, especially for the 5′ seed region of the miRNA, as demonstrated in Figure 4 with closely related miRNAs that differ by only one nucleotide at the 5′ end—the highly conserved seed region of homologous miRNAs [1]. Synthetic artificial targets of hsa-miR-17 and hsa-miR-106a were tested against corresponding TaqMan Advanced miRNA Assays and compared with another commercially available assay. Despite published claims of the other supplier's assay having minimal cross-reactivity, Figure 4 shows high cross-reactivity (105% relative detection) of the hsa-miR-17 target with the hsa-miR-106a assay, whereas the corresponding TaqMan Advanced miRNA Assay produced only 1% relative detection. This unique ability of TaqMan Advanced miRNA Assays to discriminate between highly similar miRNAs can be extremely powerful for distinguishing the biological roles of highly similar miRNAs.

A

miRNA	Sequence
hsa-miR-17	CAA AGU GCU UAC AGU GCA GGU AG
hsa-miR-106a-5p	AAA AGU GCU UAC AGU GCA GGU AG
	*

B

	Synthetic template			
	TaqMan Advanced miRNA Assays		Supplier E assays	
Assay	hsa-miR-17	hsa-miR-106a	hsa-miR-17	hsa-miR-106a
hsa-miR-17	100%	0%	100%	2%
hsa-miR-106a	1%	100%	105%	100%

Figure 4. Assay specificity with miRNA sequence similarity at the 5′ end. TaqMan Advanced miRNA Assays have a unique ability to discriminate between miRNAs differing in sequence at the 5′ end, denoted by the asterisk (*), as compared with published and in-house data of another commercially available assay. **(A)** The sequence of two closely related miRNAs, which differ by one nucleotide at the 5′ end. **(B)** Percent relative cross-reactivity for other supplier's miRNA assays and TaqMan Advanced miRNA Assays.

Reproducible with a 6-log dynamic range—precision from as little as 60 target copies

Applied Biosystems™ TaqMan® MicroRNA Assays are considered the gold standard for quantifying miRNAs by real-time PCR, and TaqMan Advanced miRNA Assays together with the TaqMan Advanced miRNA cDNA Synthesis Kit continue this reputation, with superior sensitivity and precision (Figure 5). Quadruplicate reactions with a synthetic hsa-miR-378 miRNA target exhibit excellent precision over a linear dynamic range of 6 logarithmic units. The assay accurately detects as few as 60 copies of target miRNA in the most dilute sample. The improved reproducibility of the TaqMan Advanced miRNA Assay system enables the detection of lower amounts of target miRNA and allows for better discrimination with fewer replicates required.

Excellent experimental reproducibility is accomplished using a range of synthetic oligonucleotide, nonmammalian spike-in controls that are detected with specially designed TaqMan Advanced miRNA Assays (Table 1). These spike-in controls facilitate the data normalization required to correct for technical and procedural variations particularly inherent in serum and plasma samples.

Universal amplification across a range of targets

TaqMan Advanced miRNA chemistry has no inherent bias across the ligation or miR-Amp steps, resulting in true universal amplification and superior sensitivity, regardless of the target miRNA. The workflow facilitates the addition of a poly(A) tail on the 3′ end and the ligation of an adapter on the 5′ end, yielding universal sequences that are recognized by the miR-Amp universal primers. These universal primers amplify all of the cDNA in the sample during the miR-Amp step, providing increased sensitivity without introducing amplification bias.

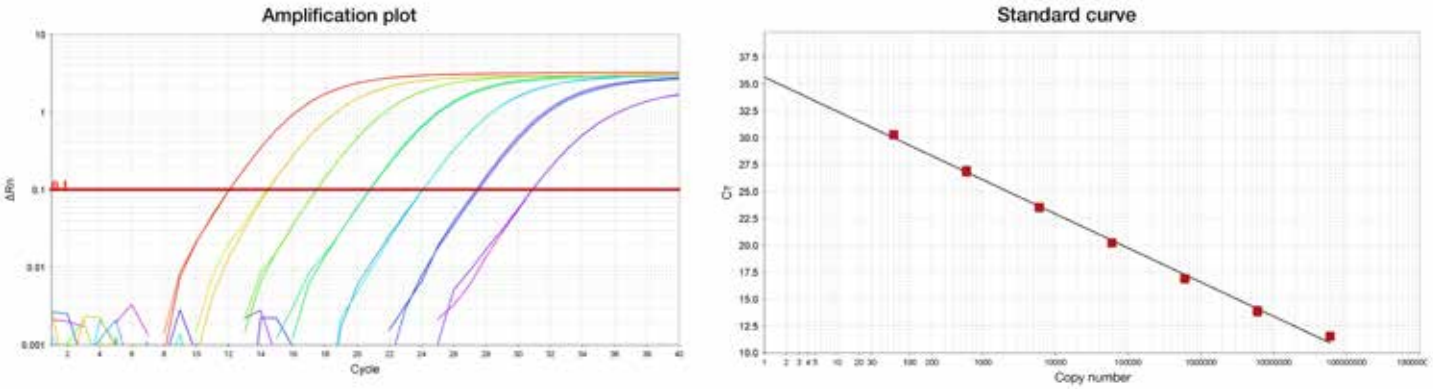

Figure 5. Dynamic range and sensitivity. A TaqMan Advanced miRNA Assay detects a synthetic hsa-miR-378 miRNA target down to 60 copies with a 6–logarithmic unit dynamic range.

Table 1. A set of 7 nonmammalian miRNAs is available for use as spike-in controls, to normalize sample input amount for difficult samples such as serum and plasma.

Control type	Gene ID/miRBase ID (v22)	Assay ID	Product name	Target sequence
Exogenous	ath-miR159a	478411_mir	ath-miR159a	UUUGGAUUGAAGGGAGCUCUA
Exogenous	cel-lin-4	478289_mir	cel-lin-4-5p	UCCCUGAGACCUCAAGUGUGA
Exogenous	cel-miR-2	478291_mir	cel-miR-2-3p	UAUCACAGCCAGCUUUGAUGUGC
Exogenous	cel-miR-238	478292_mir	cel-miR-238-3p	UUUGUACUCCGAUGCCAUUCAGA
Exogenous	cel-miR-39	478293_mir	cel-miR-39-3p	UCACCGGGUGUAAAUCAGCUUG
Exogenous	cel-miR-54	478410_mir	cel-miR-54-3p	UACCCGUAAUCUUCAUAAUCCGAG
Exogenous	cel-miR-55	478295_mir	cel-miR-55-3p	UACCCGUAUAAGUUUCUGCUGAG

A range of products for a range of situations

TaqMan Advanced miRNA Assays provide even more options to support your miRNA research and application needs. Standard TaqMan MicroRNA Assays continue to offer broad miRBase coverage and gold-standard specificity over a wide range of predesigned assays and formats, while TaqMan Advanced miRNA Assays use a universal RT step and provide superior sensitivity for biological samples. Use Table 2 to help select the TaqMan® miRNA product that is best suited to your need.

Reference

1. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116:281–297.

Table 2. TaqMan miRNA assay selection guide.

	TaqMan MicroRNA Assays	TaqMan Advanced miRNA Assays
Description	TaqMan MicroRNA Assays employ a novel target-specific stem-loop primer during cDNA synthesis that produces a template for real-time PCR	TaqMan Advanced miRNA Assays employ a universal RT step for a streamlined workflow and a universal miR-Amp step to enable highly sensitive detection by real-time PCR
RT chemistry	miRNA-specific RT	Universal RT
Throughput	Best for 1–10 targets	Best for >10 targets
Coverage	205 species available; coverage for miRBase v.21	All human, mouse, and rat miRNAs; coverage for miRBase v.21
Formats	Available in individual tubes, TaqMan® array cards and plates, and OpenArray™ formats	Available in individual tubes; inquire for custom plating options

Ordering information

Product	Quantity	Cat. No.
TaqMan Advanced miRNA cDNA Synthesis Kit	50 reactions	A28007
TaqMan Advanced miRNA Assays	250 qPCR reactions (20 µL)	A25576

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Find out more at **thermofisher.com/advancedmirna**

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