# Performance runs on TaqMan

## TaqMan Advanced miRNA Assays are available in flexible formats

With over 25 years of proven expertise and performance, we offer a fully integrated suite of gold-standard assays and tools to help you achieve maximum productivity and high-quality results from your qPCR research. Whether it's using convenient formats, obtaining the essential costeffective answers that you want, accurate results from limited samples, or high-throughput efficiency, we've got you covered.

Applied Biosystems<sup>™</sup> TaqMan<sup>®</sup> Advanced miRNA Assays employ a universal reverse transcription (RT) step for a streamlined workflow and a universal miR-Amp step to enable highly sensitive detection by real-time PCR.

#### **Key features**

- Universal RT—one RT step for all 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 ng or 2 µL of purified total RNA from plasma or serum
- Versatile—compatible with total RNA from tissue, FFPE tissues, and biofluids, including serum and plasma



#### Introduction

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 roles 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<sup>™</sup> TaqMan<sup>®</sup> Advanced miRNA cDNA Synthesis Kit have been 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.



Figure 1. The TaqMan Advanced miRNA Assay workflow.

	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 2. 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 (\*).

TagMan Advanced miRNA Assays are then used to quantitate each miRNA target by gPCR. Drawing from the proprietary Applied Biosystems™ bioinformatics assay design pipeline, TagMan Advanced miRNA Assays contain 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, TagMan Advanced miRNA Assays provide up to 6 logarithmic units of dynamic range using as little as 1 ng of total RNA from tissue or 2 µL of purified total RNA 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 TaqMan Advanced MicroRNA Assay specificity

When closely related mature miRNAs differ by as little as one base, it's important to use tools with the power to discriminate between these highly similar targets. The specificity of our assays is demonstrated using a panel of closely related let-7 miRNAs (Figure 2). Each assay was tested individually against synthetic miRNAs for members of the let-7 family, where differences in C<sub>t</sub> values were used to calculate the percent of relative detection. There is minimal or no cross-reactivity between each member of the let-7 family.

#### 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 3). 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.

#### Choose the right format that fits your research

The Applied Biosystems<sup>™</sup> TaqMan<sup>®</sup> Advanced miRNA single-tube assays, plates and array cards employ miRBase v.21 coverage and one universal RT step. They are available in a variety of formats, ranging from fixed and custom content to address your unique research needs. Use Table 1 to help select the TaqMan miRNA product that is best suited to your needs.

#### Table 1. TaqMan miRNA assay selection guide.

	TaqMan MicroRNA Assays		TaqMan Advanced miRNA Assays		
Description	Applied Biosystems <sup>™</sup> TaqMan <sup>®</sup> 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.20		All human, mouse, and rat miRNAs; coverage for miRBase v.21		
Formats	Available in individual tubes, TaqMan array cards and plates, and OpenArray <sup>™</sup> formats		Available in individual tubes, TaqMan array cards and plates, and OpenArray formats; inquire for custom plating options		
Flexible formats					
TaqMan Advanced miRNA single-tube assays		Conserve limited samples; requires only 1–10 ng of total RNA or equivalent, and is ideal f small number of assays			
New TaqMan <sup>®</sup> Advanced miRNA 96-well plates		Comprehensive—choose from a large collection of preplated, predesigned panels to pinpoint your specific target			
New TaqMan <sup>®</sup> Advanced miRNA array cards		Small reaction volumes and streamlined workflow enable profiling up to 754 miRNA targets from as little as 1 ng of total RNA on TaqMan Advanced miRNA array cards			
New TaqMan <sup>®</sup> Advanced miRNA Open Array		Run 3 samples per Applied Biosystems <sup>®</sup> QuantStudio <sup>®</sup> 12K Flex OpenArray Plate translating up to 799 assays per sample (up to 752 unique assays)			







Data showing superior reproducibility

**Figure 3. 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.

Figure 4. Reproducibility of C<sub>t</sub> values on TaqMan Advanced miRNA Human A Panel Cards. Correlation plot comparing C<sub>t</sub> values between 2 independent technical replicates. Each TaqMan Array card was processed with 10 ng of total brain RNA; data shows remarkable concordance of C<sub>t</sub> values across >300 miRNA targets.

## applied biosystems



## Innovative qPCR analysis tools available as desktop software and/or cloud apps

The Applied Biosystems<sup>™</sup> qPCR analysis modules are intuitive, cloud-based secondary data analysis solutions that combine multiple data sets in one convenient place, making it easier for you to view, store, and analyze your qPCR data.

#### **Outlier Wheel feature**

The Applied Biosystems<sup>™</sup> Outlier Wheel is a data review tool that can greatly simplify the quality control review of very large data sets, especially data generated by TaqMan Array Cards and OpenArray experiments. When a sample, target, or plate data set consists of thousands of data points, you can use the Outlier Wheel to organize and review the data set for irregular amplification.

#### **Ordering information**

Product	Quantity	Cat. No.
Plates		
TaqMan Advanced miRNA Human A and B 96-well Plates, Standard	8	A31810
TaqMan Advanced miRNA Human A 96-well Plates, Standard	4	A31811
TaqMan Advanced miRNA Human B 96-well Plates, Standard	4	A31812
TaqMan Advanced miRNA Human Endogenous Controls 96-well Plate, Standard	1	A34642
TaqMan Advanced miRNA Human Serum/Plasma 96-well Plates, Standard	2	A31813
TaqMan Advanced miRNA Human A and B 96-well Plates, Fast	8	A31875
TaqMan Advanced miRNA Human A 96-well Plates, Fast	4	A31876
TaqMan Advanced miRNA Human B 96-well Plates, Fast	4	A31877
TaqMan Advanced miRNA Human Endogenous Controls 96-well Plate, Fast	1	A34643
TaqMan Advanced miRNA Human Serum/Plasma 96-well Plates, Fast	2	A31878
Cards		
TaqMan Advanced miRNA Human A and B Cards	1	A31805
TaqMan Advanced miRNA Human A Card	1	A34714
TaqMan Advanced miRNA Human B Card	1	A34715
TaqMan Advanced miRNA Human Engodenous Controls Card	1	A34716
TaqMan Advanced miRNA Human Serum/Plasma Card	1	A34717
Open array		
TaqMan OpenArray Human Advanced MicroRNA Panel	1	A32710
Related products		
TaqMan Advanced miRNA cDNA Synthesis Kit	50 rxns	A28007
TaqMan Fast Advanced Master Mix	1 x 1 mL	4444556

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