

TaqMan® MicroRNA Assays
The Gold Standard

Validation of microRNA (miRNA) expression is a critical component in the workflow for any miRNA research. Here we evaluate miRNA quantification products from various vendors.

Currently available PCR-based methods require a basic two-step RT-PCR process.

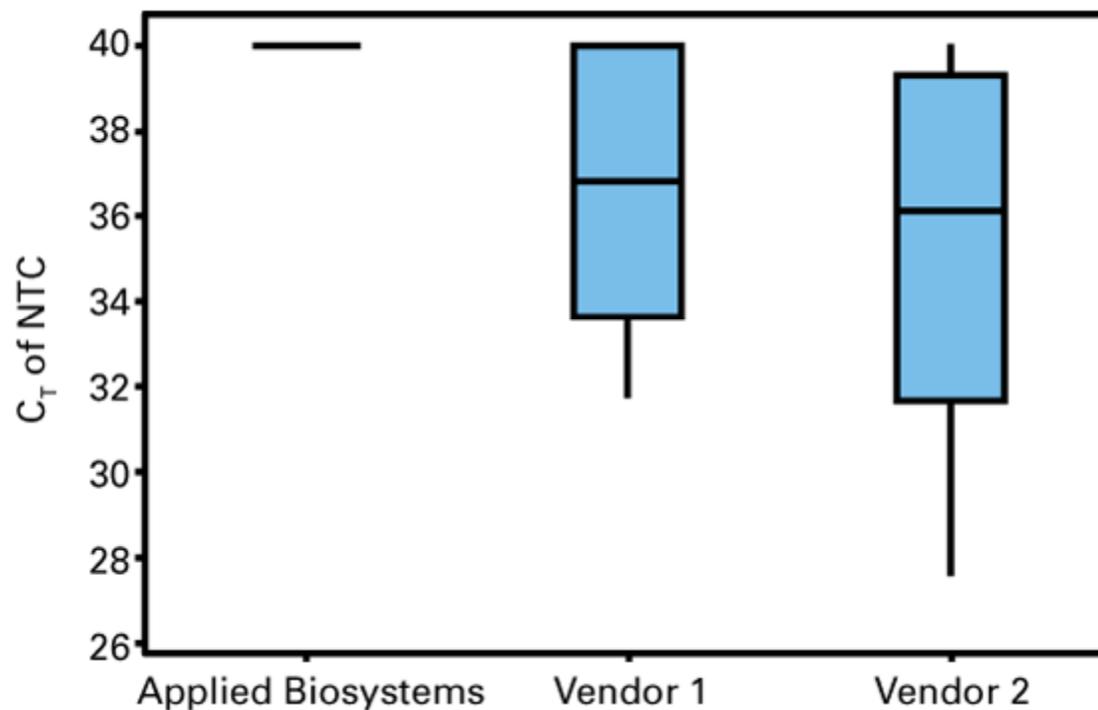
In this study, vendors employing one of each of the two general RT methods are chosen for comparison:

- Specific RT (Vendor 1)
- Nonspecific RT (Vendor 2)

The following parameters were evaluated:

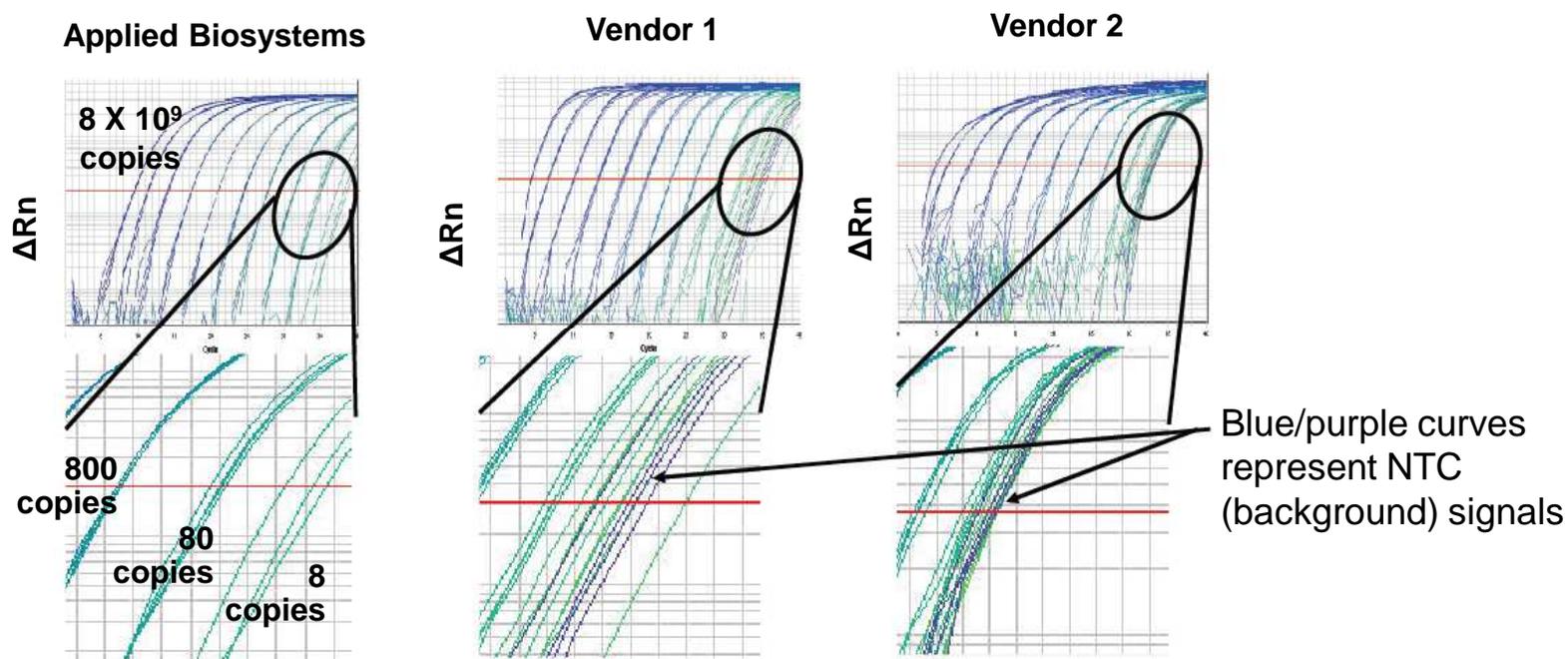
- No-template controls (NTC—background signal)
- Linear dynamic range
- Sensitivity
- Cross-specificity between closely related miRNAs
- Specificity between mature and precursor sequences
- Two-fold expression change

The gold standard begins with providing minimal background and variation, and a C_T of 40 for no-template controls.



Capturing significant noise down to a C_T of 32 is detrimental to validation. Values at that level may fall within the same range as miRNAs expressed at lower levels and abundant miRNAs measured at low sample input.

The essence of TaqMan[®] MicroRNA Assays as a gold standard lies in their broad dynamic range and high sensitivity.



Applied Biosystems TaqMan[®] MicroRNA Assays have a linear dynamic range of 9 log units and can reliably detect as few as 10 copies of synthetic miRNAs. Other methods show high variation and background signal with the same amount of RNA. Compromising sensitivity increases the risk of inefficient detection of miRNAs at various expression levels.

TaqMan[®] MicroRNA Assays use four oligonucleotides (TaqMan[®] probe, RT primer, and forward and reverse PCR primers) to help achieve high specificity.

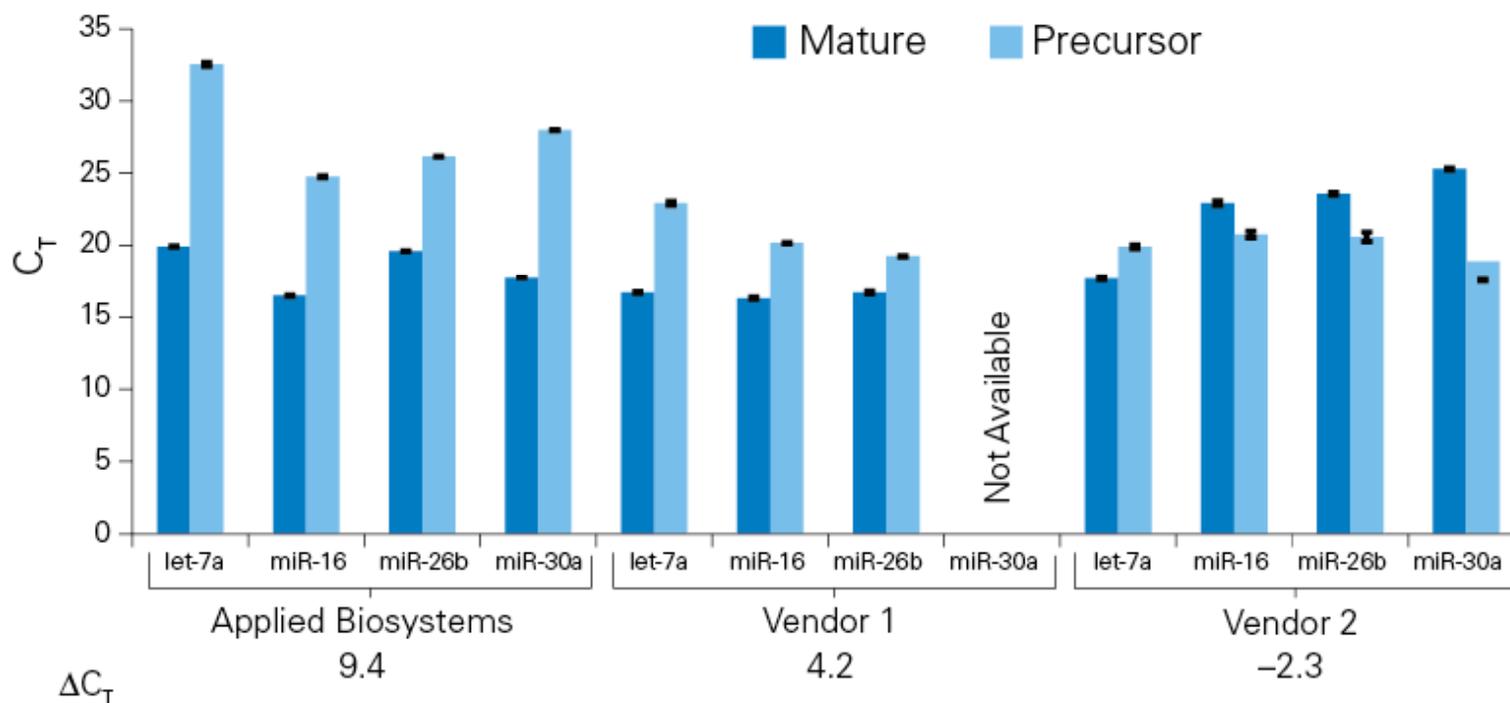
Applied Biosystems					Vendor 1					Vendor 2				
	Synthetic Template					Synthetic Template					Synthetic Template			
Assay	let-7a	let-7b	let-7c	let-7d	Assay	let-7a	let-7b	let-7c	let-7d	Assay	let-7a	let-7b	let-7c	let-7d
let-7a	1.00	0.00	0.03	0.01	let-7a	1.00	0.04	0.51	0.03	let-7a	1.00	0.00	0.00	0.01
let-7b	0.00	1.00	0.07	0.00	let-7b	0.00	1.00	0.23	0.00	let-7b	0.00	1.00	0.00	0.00
let-7c	0.00	0.02	1.00	0.00	let-7c	0.02	0.83	1.00	0.00	let-7c	0.00	0.02	1.00	0.00
let-7d	0.03	0.00	0.01	1.00	let-7d	Assay not available				let-7d	0.24	0.00	0.00	1.00

hsa-let-7a UGAGGUAGUAGGUUGUAUAGUU
 hsa-let-7b UGAGGUAGUAGGUUGUGUGUU
 hsa-let-7c UGAGGUAGUAGGUUGUAUGGUU
 hsa-let-7d AGAGGUAGUAGGUUGCAUAGUU

Indicates a perfect match
 Indicates low cross-reactivity
 Indicates high cross-reactivity

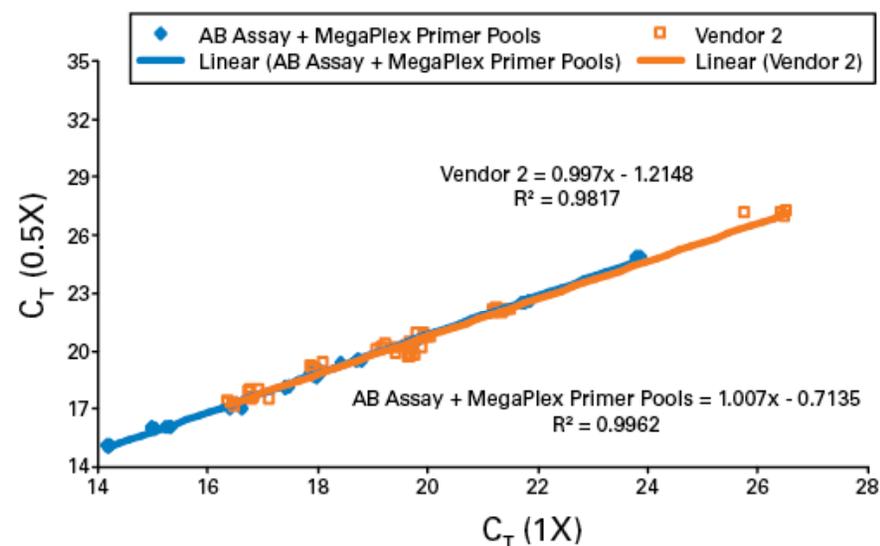
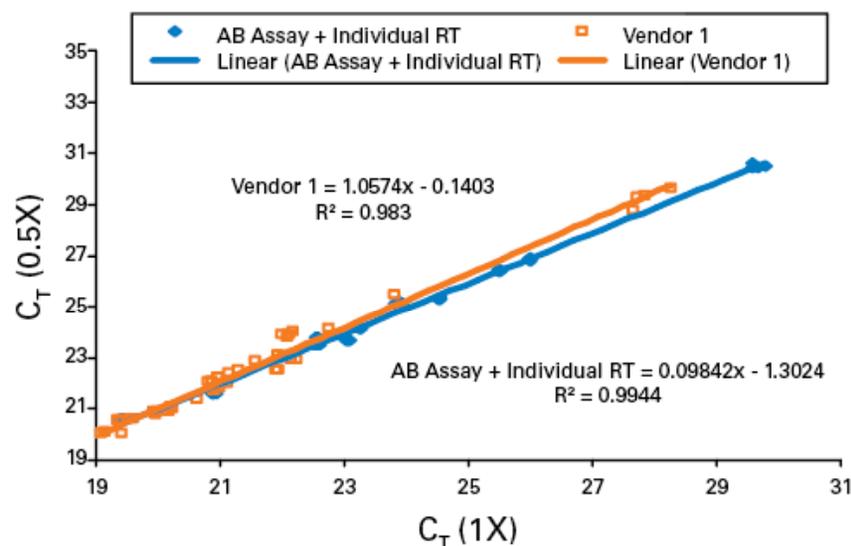
Applied Biosystems uses the same bioinformatic pipeline for all TaqMan[®] MicroRNA Assays, ensuring consistent specificity and sensitivity. Manually adjusting individual assays to increase specificity results in altered sensitivity.

The greater biological significance of mature miRNAs¹ necessitates the high level of discrimination between precursor and mature forms available with TaqMan[®] MicroRNA Assays.



1. Du, T and Zamore, PD (2005) microPrimer: the biogenesis and function of microRNA *Development* 132: 4645–4652

TaqMan[®] MicroRNA Assays enable reliable quantification of the miRNAs present in real tissue samples.



Lung samples were evaluated for expression of 12 miRNAs. TaqMan[®] assays demonstrated excellent discrimination of two-fold expression (Avg ΔC_T closest to 1) and the least variation (R^2 closest to 1). A touted benefit of nonspecific RT (Vendor 2) is the ability to simultaneously generate cDNAs for all miRNAs. For comparison to Vendor 2, Applied Biosystems Megaplex[™] RT Primer Pools were used instead of the individual RT primers.

TaqMan[®] MicroRNA Assays hold true to their promise of being the gold standard.

With TaqMan[®] MicroRNA Assays:

- You can be confident that you have a clean assay with minimal background noise
- You can achieve unrivaled linear dynamic range and unsurpassed sensitivity, detecting down to 10 copies miRNA
- You can discriminate between closely related miRNAs, and be sure that only biologically active mature miRNAs are detected
- You get reliable detection and quantification of miRNAs present in your sample

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Materials and Methods

- NTC data were obtained for 15 assays chosen to represent a challenging set (below—Sanger 10.0).
- For dynamic range and sensitivity tests, data are displayed for the let-7a assay. These were performed at 10 different concentrations (green curves) in addition to the NTC (in blue/purple). Four replicate reactions were performed at each concentration (see Slide 4).
- For the mature/precursor specificity tests, 4 replicate assays were performed for each miRNA tested.
- For the 2-fold expression change, 12 miRNAs were evaluated (see below, without 7i, RNU44, and RNU48). Total RNA input was within the recommended range—1 ng/μL (1X) and 0.5 ng/μL (0.5X) for individual RT reactions, or 20 ng/μL (1X) and 10 ng/μL (0.5X) for multiplexed RT reactions.
- All other methods were performed according to the vendors' protocols and recommendations.

let-7a	let-7b	let-7c	let-7d	let-7e	let-7f	let-7g	let-7i	miR-16	miR-23a	miR-23b	miR-148b	miR-194	RNU44	RNU48
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A license to perform the patented 5' Nuclease Process for research is obtained by the purchase of (i) both Licensed Probe and Authorized 5' Nuclease Core Kit, (ii) a Licensed 5' Nuclease Kit, or (iii) license rights from Applied Biosystems.

The TaqMan[®] MicroRNA Assay contains Licensed Probe. Use of this product is covered by US patent claims and patent claims outside the US. The purchase of this product includes a limited, non-transferable immunity from suit under patent claims for using only this amount of product for the purchaser's own internal research. Separate purchase of an Authorized 5' Nuclease Core Kit would convey rights under the applicable claims of US patents and patent claims outside the United States, which claim 5' nuclease methods. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by etoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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