ThermoFisher SCIENTIFIC

Sample prep

Analysis of multiple miRNA targets using the TaqMan Fast Advanced Cells-to-C_T Kit and TaqMan Advanced miRNA Assays

Summary

- **Fast**—demonstrated preparation of miRNA in 8 minutes using the Invitrogen™ TaqMan® Fast Advanced Cells-to-C_T™ Kit
- **Simple protocol**—one RT step for all Applied Biosystems™ TaqMan® Advanced miRNA Assays
- **Sensitive for small sample input**—expression of multiple miRNAs can be studied when the sample quantity is limited
- **Accurate and robust measurement of miRNA**—TaqMan Advanced miRNA Assays show high concordance with Applied Biosystems™ TaqMan® MicroRNA Assays for miRNA expression analysis

Introduction

MicroRNAs (miRNAs) are small noncoding RNA molecules that play major roles in posttranscriptional regulation of gene expression [1]. There is increased interest in researching miRNAs because of their association with human diseases [2]. Here we demonstrate a fast, sensitive, and robust method with a streamlined workflow for miRNA preparation using the TaqMan Fast Advanced Cells-to-C_T Kit. Furthermore, we highlight the TaqMan Advanced miRNA Assay and its use with the Applied Biosystems™ TaqMan® Advanced miRNA cDNA Synthesis Kit, which enables reverse transcription of all miRNA targets in one reaction. TaqMan Advanced miRNA Assays are equivalent in performance to standard TaqMan MicroRNA Assays.

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Materials and methods

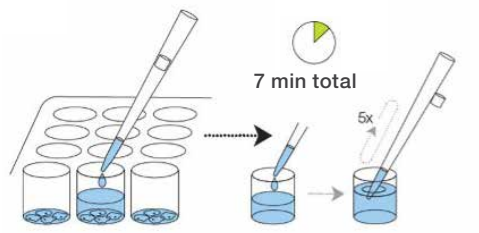
Nucleic acid preparation

Triplicate sample lysates were prepared from 10,000 HeLa or HepG2 cells using the TaqMan Fast Advanced Cells-to-C_T Kit according to the user guide. The lysis solution incubation step was increased to 8 minutes at room temperature to ensure complete lysis of cells and increase sensitivity for miRNAs (Figure 1). The cell lysates were processed using two different workflows for miRNA expression assays (Figure 2).

For the TaqMan MicroRNA Assay, duplicate reverse transcription reactions were performed on lysates using the Applied Biosystems™ TaqMan® MicroRNA Reverse Transcription Kit and the miRNA-specific stem-loop RT primer provided with the individual TaqMan MicroRNA Assay (Figure 3A). Following the reverse transcription for each miRNA, an aliquot of the reaction was used for real-time PCR in duplicate using the miRNA-specific TaqMan probe and primer set.

For the TaqMan Advanced miRNA Assay, all mature miRNAs in lysates were modified by the addition of a poly(A) tail (3′) and an adaptor (5′), reverse transcribed, and then amplified in a single reaction using the TaqMan Advanced miRNA cDNA Synthesis Kit (Figure 3B). This enabled amplification of the sample for downstream real-time PCR in a single universal reaction. The resulting high amount of cDNA (miR-Amp reaction) is ideal for all TaqMan Advanced miRNA Assays. In this study, eight different miRNAs (Table 1) were tested using both assay workflows shown in Figure 2.

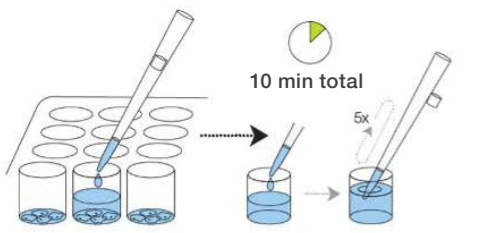
A



Lysis Solution + Stop Solution

1. Remove culture medium. Wash cells with PBS.
2. (Optional) Dilute DNase into Lysis Solution.
3. Add Lysis Solution and mix 5 times.
4. Incubate for 5 min at room temp (19–25°C).
5. Add stop solution and mix 5 times.
6. Incubate for 2 min at room temp.

B



Lysis Solution + Stop Solution

1. Remove culture medium. Wash cells with PBS.
2. (Optional) Dilute DNase into Lysis Solution.
3. Add Lysis Solution and mix 5 times.
4. Incubate for 8 min at room temp (19–25°C).
5. Add stop solution and mix 5 times.
6. Incubate for 2 min at room temp.

Figure 1. Sample preparation for miRNA detection using the TaqMan Fast Advanced Cells-to-C_T Kit. (A) Standard protocol of the TaqMan Fast Advanced Cells-to-C_T Kit for miRNA detection. **(B)** Increase of lysis time to 8 minutes for increased sensitivity of miRNA detection.

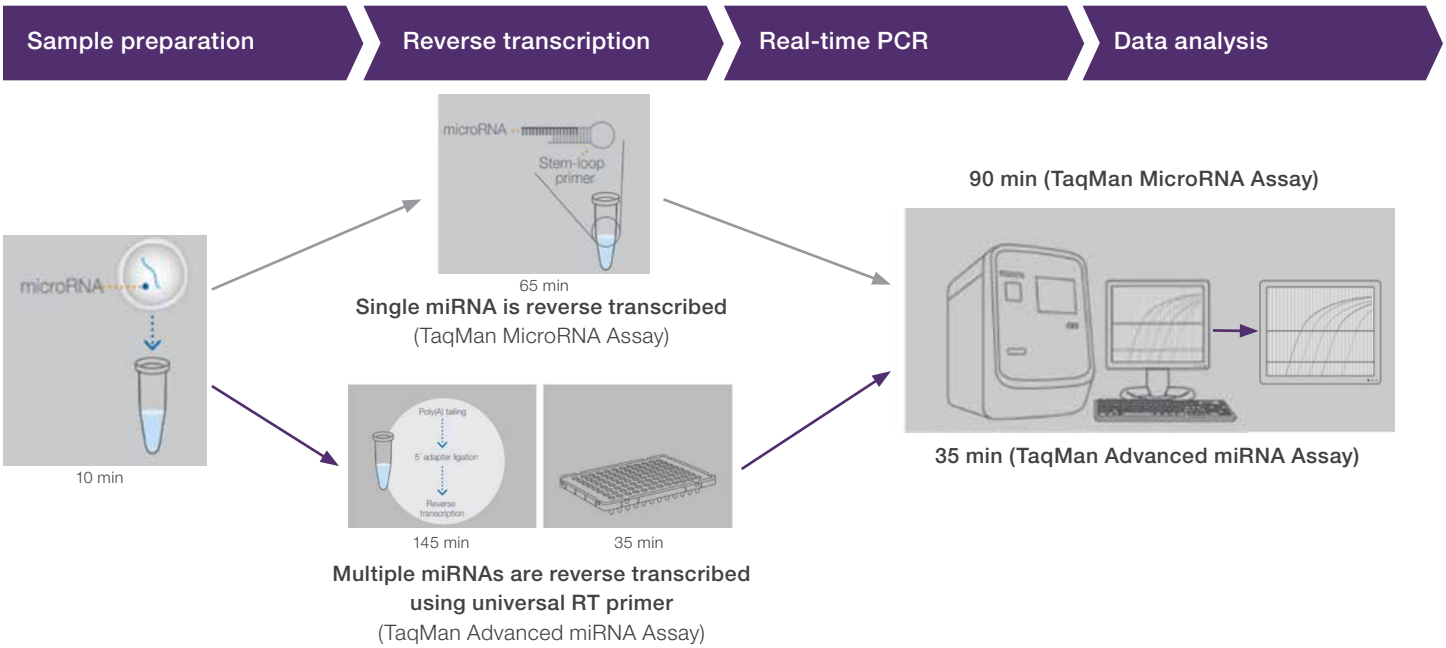


Figure 2. Workflows for the TaqMan MicroRNA Assay and TaqMan Advanced miRNA Assay, compared. The TaqMan MicroRNA Assay requires individual reverse transcription of each miRNA of interest, whereas the TaqMan Advanced miRNA Assay utilizes a universal RT primer, which is advantageous for studying multiple miRNAs from the same sample.

miRNA detection by real-time PCR

miRNA expression was evaluated by real-time PCR. For TaqMan Advanced miRNA Assays, a 5 µL sample (1:10 dilution of the miR-Amp reaction) was used with TaqMan Fast Advanced Master Mix and the TaqMan Advanced miRNA Assay in a 20 µL reaction. Real-time PCR was performed using the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System (384-well block) and the following cycling conditions: 1 cycle of enzyme activation at 95ºC for 20 seconds, 40 cycles of amplification at 95ºC for 1 second and 60ºC for 20 seconds. For TaqMan MicroRNA Assays, a 1.33 µL sample from the reverse transcription step was used with TaqMan Fast Advanced Master Mix and a TaqMan MicroRNA Assay in a 20 µL reaction. Real-time PCR was performed using the QuantStudio 5 Real-Time PCR System (384-well block) and the following cycling conditions: 1 cycle of enzyme activation at 95ºC for 10 minutes, 40 cycles of amplification at 95ºC for 15 seconds and 60ºC for 1 minute. Real-time PCR reactions were carried out in duplicate for each of the reverse transcription reactions, and data were analyzed by setting the threshold at 0.1.

Results

The TaqMan Advanced miRNA Assay and the TaqMan MicroRNA Assay have equivalent performance (Figure 4). The TaqMan Advanced miRNA Assay utilizes a protocol with simultaneous reverse transcription of all mature miRNAs in a single reaction. This allows for more streamlined reverse transcription of samples, analysis of multiple miRNAs from a single sample, and ease of use for the researcher.

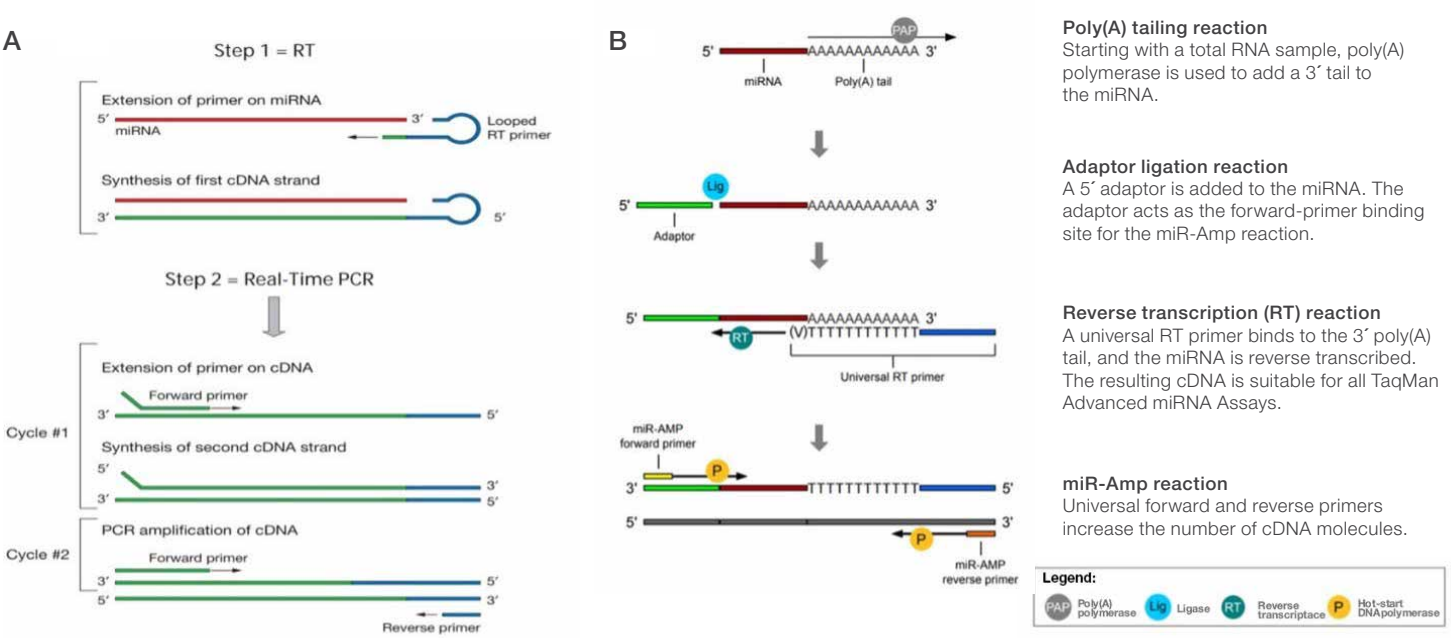


Figure 3. Comparison of miRNA assays. (A) The TaqMan MicroRNA Assay utilizes a simple two-step protocol that requires a novel target-specific stem-loop primer for cDNA synthesis to produce a template that is used subsequently for real-time PCR with the corresponding TaqMan probe and primer set. **(B)** The TaqMan Advanced miRNA Assay utilizes a universal RT step for a streamlined workflow, and a universal miR-Amp step to enable highly sensitive detection by real-time PCR.

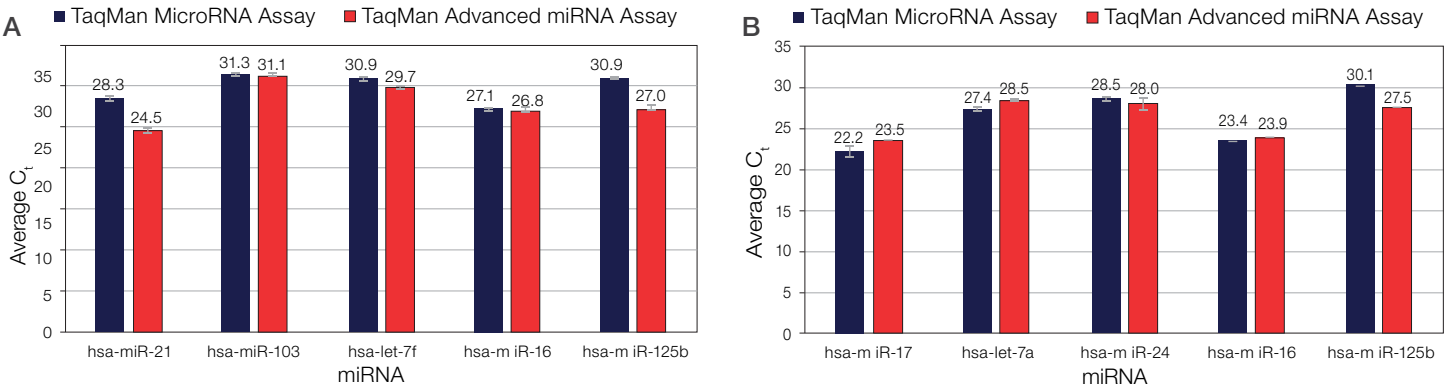


Figure 4. Sensitivity of the TaqMan Advanced miRNA Assay. The TaqMan Advanced miRNA Assay and TaqMan MicroRNA Assay show equivalent performance. Average C_t values are shown from reactions prepared with the TaqMan Fast Advanced Cells-to-C_T Kit, using **(A)** 10,000 HeLa cells and **(B)** 10,000 HepG2 cells.

Table 1. miRNA and corresponding assay ID for each TaqMan MicroRNA Assay and TaqMan Advanced miRNA Assay.

miRNA	Assay ID for TaqMan MicroRNA Assay (Cat. No. 4427975)	Assay ID for TaqMan Advanced miRNA Assay (Cat. No. A25576)	Mature miRNA sequence
hsa-miR-21	000397	477975_mir	UAGCUUAUCAGACUGAUGUUGA
hsa-miR-103	000439	478253_mir	AGCAGCAUUGUACAGGGCUAUGA
hsa-let-7f	000382	478578_mir	UGAGGUAGUAGAUUGUAUAGUU
hsa-miR-16	000391	477860_mir	UAGCAGCACGUAAAUAUUGGCG
hsa-miR-125b	000449	477885_mir	UCCCUGAGACCCUAACUUGUGA
hsa-miR-24	000402	477992_mir	UGGCUCAGUUCAGCAGGAACAG
hsa-miR-17	002308	478447_mir	CAAAGUGCUUACAGUGCAGGUAG
hsa-let-7a	000377	478575_mir	UGAGGUAGUAGGUUGUAUAGUU

Conclusion

The TaqMan Fast Advanced Cells-to-C_T Kit can be used to efficiently prepare samples for miRNA expression studies. Multiple miRNA targets can be studied from a single RT reaction using the TaqMan Advanced miRNA cDNA Synthesis Kit and TaqMan Advanced miRNA Assay. The TaqMan Advanced miRNA Assay demonstrates performance equivalent to the TaqMan MicroRNA Assay, with an easier workflow when analyzing various miRNAs from a single sample. The combination of fast sample preparation and ease of use for miRNA detection is a powerful tool to speed up your research.

References

1. O'Brien J, Hayder H, Zayed Y, Peng C (2018) Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Front Endocrinol* 9:402. doi: 10.3389/fendo.2018.00402
2. Wang J, Chen J, Sen S (2016) MicroRNA as biomarkers and diagnostics. *J Cell Physiol* 231:25–30. doi: 10.1002/jcp.25056

Ordering information

Product	Quantity	Cat. No.
TaqMan Fast Advanced Cells-to-C_T Kit	40 reactions	A35374
	100 reactions	A35377
	400 reactions	A35378
TaqMan MicroRNA Reverse Transcription Kit	200 reactions	4366596
	1,000 reactions	4366597
TaqMan Advanced miRNA cDNA Synthesis Kit	50 reactions	A28007
TaqMan MicroRNA Assay (inventoried; small scale)	50 RT reactions, 150 PCR reactions	4427975
TaqMan MicroRNA Assay (made-to-order; medium scale)	750 RT reactions, 750 PCR reactions	4440887
TaqMan MicroRNA Assay (made-to-order; large scale)	2,900 RT reactions, 2,900 PCR reactions	4440888
TaqMan Advanced miRNA Assay	250 reactions	A25576
TaqMan Fast Advanced Master Mix	1 x 1 mL	4444556
	1 x 5 mL	4444557
	2 x 5 mL	4444963
	5 x 5 mL	4444964
	10 x 5 mL	4444965
	1 x 50 mL	4444558

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