TaqMan® Advanced miRNA Assays

TaqMan® Array Cards

Pub. No. MAN0016123 **Rev.** B.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TaqMan® Advanced miRNA Assays User Guide (TaqMan® Array Cards)* (Pub. No. MAN0016122). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This Quick Reference is intended as a benchtop reference for experienced users of TaqMan® Advanced miRNA Assays and the TaqMan® Advanced miRNA cDNA Synthesis Kit (Cat. No. A28007; sold separately). For detailed instructions, supplemental procedures, and troubleshooting, see the *TaqMan® Advanced miRNA Assays User Guide (TaqMan® Array Cards)* (Pub. No. MAN0016122).

Prepare cDNA templates

Procedural guidelines

Guidelines for RNA input

- Prepare samples using a total RNA isolation method that preserves small RNAs.
- For tissue samples: Use 1–10 ng of total RNA per reaction.
- For blood, serum, or plasma samples: Use 2 µL of sample eluent (from the sample isolation procedure) per reaction. If RNA can be quantified, use 1–10 ng of total RNA per reaction.
- For optimal reverse transcription, input RNA should be:
  - Free of inhibitors of reverse transcription (RT) and PCR
  - Dissolved in PCR-compatible buffer
  - Free of RNase activity
  - Nondenatured total RNA

Guidelines for preparing cDNA templates

- Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes, then include 10% overage.
- See your instrument user guide for detailed instructions about using plates, tubes, or strip tubes.

Perform the poly(A) tailing reaction

1. Thaw samples and cDNA synthesis reagents on ice, gently vortex, then centrifuge briefly.

   IMPORTANT! The 50% PEG 8000 reagent must be at room temperature for the adaptor ligation reaction (next section).

2. In a 1.5-mL microcentrifuge tube, prepare sufficient Poly(A) Reaction Mix for the required number of reactions according to the following table.

Component	1 Rxn	4 Rxns[1]	10 Rxns[1]
10X Poly(A) Buffer	0.5 µL	2.2 µL	5.5 µL
ATP	0.5 µL	2.2 µL	5.5 µL
Poly(A) Enzyme	0.3 µL	1.3 µL	3.3 µL
RNase-free water	1.7 µL	7.5 µL	18.7 µL
Total Poly(A) Reaction Mix volume	**3.0 µL**	**13.2 µL**	**33 µL**

[1] Volumes include 10% overage.

3. Vortex the Poly(A) Reaction Mix, then centrifuge briefly.
4. Add 2 µL of sample to each well of a reaction plate or each reaction tube.
5. Add 3 µL of Poly(A) Reaction Mix to each well or tube.

   The total volume should be 5 µL per well or tube.
6. Seal the reaction plate or tubes, then vortex briefly to thoroughly mix the contents.
7. Centrifuge the reaction plate or tubes briefly to spin down the contents and eliminate air bubbles.
8. Place the reaction plate or tubes into a thermal cycler, then incubate using the following settings and standard cycling:

Step	Temperature	Time
Polyadenylation	37°C	45 minutes
Stop reaction	65°C	10 minutes
Hold	4°C	Hold

Proceed immediately to the adaptor ligation reaction (next section).

Perform the adaptor ligation reaction

1. In a 1.5-mL microcentrifuge tube, prepare sufficient Ligation Reaction Mix for the required number of reactions according to the following table.

Component	1 Rxn	4 Rxns[1]	10 Rxns[1]
5X DNA Ligase Buffer	3 µL	13.2 µL	33 µL
50% PEG 8000[2]	4.5 µL	19.8 µL	49.5 µL
25X Ligation Adaptor	0.6 µL	2.6 µL	6.6 µL
RNA Ligase	1.5 µL	6.6 µL	16.5 µL
RNase-free water	0.4 µL	1.8 µL	4.4 µL
Total Ligation Reaction Mix volume	**10 µL**	**44 µL**	**110 µL**

[1] Volumes include 10% overage.
[2] 50% PEG 8000 is very viscous, follow the Important statement below to ensure accurate pipetting.

IMPORTANT! For accurate pipetting of 50% PEG 8000:

- Use 50% PEG 8000 at room temperature.
- Aspirate and dispense solution slowly.

2. Vortex the Ligation Reaction Mix, then centrifuge briefly.
3. Transfer 10 µL of the Ligation Reaction Mix to each well of the reaction plate or each reaction tube containing the poly(A) tailing reaction product.

   The total volume should be 15 µL per well or tube.
4. Seal the reaction plate or tubes, then vortex briefly or shake (1,900 rpm for 1 minute with an Eppendorf™ MixMate™ (Cat. No. 21-379-00)) to thoroughly mix the contents.

IMPORTANT! If vortexing, watch for a swirling motion of the adaptor ligation reaction to ensure proper mixing. Proper mixing is necessary for efficient ligation.

5. Centrifuge the reaction plate or tubes briefly to spin down the contents.
6. Place the reaction plate or tubes into a thermal cycler, then incubate using the following settings and standard cycling:

Step	Temperature	Time
Ligation	16°C	60 minutes
Hold	4°C	Hold

Proceed immediately to the reverse transcription (RT) reaction (next section).

Perform the reverse transcription (RT) reaction

1. In a 1.5-mL microcentrifuge tube, prepare sufficient RT Reaction Mix for the required number of reactions according to the following table.

Component	1 Rxn	4 Rxns[1]	10 Rxns[1]
5X RT Buffer	6 µL	26.4 µL	66 µL
dNTP Mix (25 mM each)	1.2 µL	5.3 µL	13.2 µL
20X Universal RT Primer	1.5 µL	6.6 µL	16.5 µL
10X RT Enzyme Mix	3 µL	13.2 µL	33 µL
RNase-free water	3.3 µL	14.5 µL	36.3 µL
Total RT Reaction Mix volume	**15 µL**	**66 µL**	**165 µL**

[1] Volumes include 10% overage.

2. Vortex the RT Reaction Mix, then centrifuge briefly.
3. Transfer 15 µL of the RT Reaction Mix to each well of the reaction plate or each reaction tube containing the adaptor ligation reaction product.

   The total volume should be 30 µL per well or tube.
4. Seal the reaction plate or tubes, then vortex briefly to thoroughly mix the contents.
5. Centrifuge the reaction plate or tubes briefly to spin down the contents.
6. Place the reaction plate or tubes into a thermal cycler, then incubate using the following settings and standard cycling:

Step	Temperature	Time
Reverse transcription	42°C	15 minutes
Stop reaction	85°C	5 minutes
Hold	4°C	Hold

Proceed to the miR-Amp reaction (next section) or store the RT reaction product at –20°C for up to 2 months.

Perform the miR-Amp reaction

1. In a 1.5-mL microcentrifuge tube, prepare sufficient miR-Amp Reaction Mix for the required number of reactions according to the following table.

Component	1 Rxn	4 Rxns[1]	10 Rxns[1]
2X miR-Amp Master Mix	25 µL	110 µL	275 µL
20X miR-Amp Primer Mix	2.5 µL	11 µL	27.5 µL
RNase-free water	17.5 µL	77 µL	192.5 µL
Total miR-Amp Reaction Mix volume	**45 µL**	**198 µL**	**495 µL**

[1] Volumes include 10% overage.

2. Vortex the miR-Amp Reaction Mix, then centrifuge briefly.
3. Transfer 45 µL of the miR-Amp Reaction Mix to each well of a *new* reaction plate or reaction tube.
4. Add 5 µL of the RT reaction product to each reaction well or each reaction tube.

   The total volume should be 50 µL per well or tube.
5. Seal the reaction plate or tubes, then vortex briefly to thoroughly mix the contents.
6. Centrifuge the reaction plate or tubes briefly to spin down the contents.
7. Place the reaction plate or tubes into a thermal cycler, then incubate using the following settings, maximum ramp speed, and standard cycling:

Step	Temperature	Time	Cycles
Enzyme activation	95°C	5 minutes	1
Denature	95°C	3 seconds	14
Anneal/Extend	60°C	30 seconds	
Stop reaction	99°C	10 minutes	1
Hold	4°C	Hold	1

Proceed to performing the real-time PCR (next section) or store the undiluted miR-Amp reaction product at –20°C for up to 2 months.

Perform real-time PCR

Prepare PCR reactions

1. Prepare a 1:10 dilution of the cDNA template (the miR-Amp reaction product).

   For example, add 45 µL of the miR-Amp reaction product to 405 µL of 0.1X TE buffer.
2. Mix the TaqMan® Fast Advanced Master Mix thoroughly but gently.
3. Mix the reaction components.

Component	Volume[1]			
	1 card	2 cards	3 cards	4 cards
Diluted cDNA template	220 µL	440 µL	660 µL	880 µL
TaqMan® Fast Advanced Master Mix (2X)	440 µL	880 µL	1320 µL	1,760 µL
RNase-free water	220 µL	440 µL	660 µL	880 µL
Total volume	**880 µL**	**1,760 µL**	**2,460 µL**	**3,520 µL**

[1] Includes 10% overage.

Prepare a TaqMan® Array Card

IMPORTANT! Before preparing a TaqMan® Array Card, review *TaqMan® Advanced miRNA Assays User Guide (TaqMan® Array Cards)* (Pub. No. MAN0016122).

1. Load each fill reservoir of the card with 100 µL of prepared PCR reaction mix.
2. Centrifuge, then seal the filled card.

Set up and run the real-time PCR instrument

See the appropriate instrument user guide for detailed instructions to program the thermal-cycling conditions or to run the card.

1. Import the setup file (SDS in TXT format) into the real-time PCR instrument or software.
2. Set the properties for the run.

Property	Setting
Block	**Array Card**
Experiment type	**Comparative C_t ($\Delta\Delta C_t$)**
Reagents or Chemistry	**TaqMan® Reagents**
Cycling mode	**Fast**

Note: The default passive reference is set to ROX™ dye and should not be changed.

3. Set up the thermal protocol.

Step	Temperature	Time	Cycles
Enzyme activation	92°C	10 minutes	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

4. Confirm that the reaction volume is set to **1 µL**.
5. Load the reaction card into the real-time PCR instrument.
6. Start the run.

Analyze the results

For more information, see the appropriate documentation for your instrument.

Instrument	Data analysis settings
QuantStudio™ 7 Flex Real-Time PCR System	• Select the **Relative Threshold** algorithm setting. • Set the NOAMP Flag threshold to 0.3. • Set the C_{rt} cutoff to 32.
QuantStudio™ 12K Flex Real-Time PCR System	
ViiA™ 7 Real-Time PCR System	
7900HT Fast Real-Time PCR System	• Use the threshold (C_t) method. Relative threshold (C_{rt}) is available in the Relative Quantification app, available on the Thermo Fisher Cloud. • Use auto baseline. • Set the threshold to 0.1. The baseline and threshold values can be changed if needed. The recommended threshold is 0.1 • A cutoff of 32 is recommended.

View the C_{rt} or C_t values for each well and for each replicate group.

Analyze data that are generated with TaqMan® Advanced miRNA Assays using one of the following tools:

Software	Resource
Applied Biosystems™ real-time PCR Analysis Modules, including the Relative Quantification app	**thermofisher.com/us/en/home/cloud.html**
ExpressionSuite™ Software[1]	**thermofisher.com/us/en/home/technical-resources/software-downloads/expressionsuite-software.html**

[1] ExpressionSuite™ Software will automatically define the best threshold value.

For more information about real-time PCR, go to **thermofisher.com/qpcreducation**.

Manufacturer: Life Technologies Corporation | 6055 Sunol Blvd | Pleasanton, CA 94566

Revision history: Pub. No. MAN0016123

Revision	Date	Description
B.0	21 November 2017	• Updated instructions for mixing TaqMan® Fast Advanced Master Mix. • Updated instructions for importing assay files. • Corrected data analysis guidelines for using the relative threshold algorithm. • Changed the cutoff for data analysis using the baseline threshold algorithm from a requirement to a recommendation.
A.0	1 August 2017	New document.