TaqMan® Advanced miRNA Assays

TaqMan® Array Plates

Pub. No. MAN0016121 **Rev.** A.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TaqMan® Advanced miRNA Assays User Guide (TaqMan® Array Plates)* (Pub. No. MAN0016120). 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 Plates)* (Pub. No. MAN0016120).

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.

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, then transfer 3 µL of Poly(A) Reaction Mix to each well or tube.

   The total volume should be 5 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 and eliminate air bubbles.
7. 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

8. 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! Watch for a swirling motion of the adaptor ligation reaction to ensure proper mixing, which 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

7. 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

7. Proceed to the miR-Amp reaction (next section).

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, MAX 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

8. Proceed to performing the real-time PCR (next section).

Store the undiluted miR-Amp reaction product at –20°C for up to 2 months.

Perform real-time PCR

Procedural guidelines for performing real-time PCR

- Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes, then include 10% overage.
- For reaction volumes that are different from those detailed, scale all components proportionally.
  Reaction volumes < 10 µL are not recommended.

Prepare PCR reactions

1. Prepare a 1:10 dilution of the cDNA template (the miR-Amp reaction product).
2. Gently shake the bottle of master mix. Do not invert the bottle.
3. Remove the TaqMan® Array Plate from its packaging, centrifuge briefly, then remove the plate cover.
4. Mix the reaction components.

Component	Volume per 96-well plate[1]	
	Fast plate	Standard plate
Diluted cDNA template	264 µL	528 µL
TaqMan® Fast Advanced Master Mix (2X)	528 µL	1,056 µL
RNase-free water	264 µL	528 µL
Total reaction volume	**1,056 µL**	**2,112 µL**

[1] Includes 10% overage.

5. Add the reaction components to the wells of the reaction plate following the sample layout that is designated in your configured plate documents.
   - 10 µL per well for Fast plates
   - 20 µL per well for Standard plates
6. Seal the reaction plate with an adhesive cover, then vortex briefly to thoroughly mix the contents.
7. Centrifuge the reaction plate briefly to centrifuge the contents.

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 plate.

1. Load the reaction plate in the real-time PCR instrument.
2. Set the appropriate experiment settings and PCR thermal cycling conditions for your instrument. Select the fast cycling mode for all instruments.

Table 1 StepOnePlus™, ViiA™ 7, and QuantStudio™ systems

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

Table 2 7500 and 7500 Fast systems

Step	Temperature	Time	Cycles
Enzyme activation	95°C	20 seconds	1
Denature	95°C	3 seconds	40
Anneal / Extend	60°C	30 seconds	

3. Set the reaction volume appropriate for the reaction plate.
4. Start the run.

Analyze the results

For detailed information about data analysis, see the appropriate documentation for your instrument. Use the standard curve method or the relative quantification ($\Delta\Delta C_t$) method to analyze results.

The general guidelines for analysis include:

- View the amplification plot; then, if needed:
  - Adjust the baseline and threshold values.

    Note: A threshold value of 0.1 is recommended.
  - Remove outliers from the analysis.
- In the well table or results table, view the C_t values for each well and for each replicate group.

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

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A.0	26 October 2016	New document.