

# TaqMan® Advanced miRNA Assays

## Fixed-content TaqMan® OpenArray™ Plates

Pub. No. MAN0016125 Rev. B.0

**Note:** For safety and biohazard guidelines, see the “Safety” appendix in the *TaqMan® Advanced miRNA Assays User Guide—TaqMan® OpenArray™ Plates* (Pub. No. MAN0016124). 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® OpenArray™ Plates* (Pub. No. MAN0016124).

This Quick Reference is for the fixed-content TaqMan® OpenArray™ Plate workflow. See *TaqMan® Advanced miRNA Assays Quick Reference—Flexible-content and custom-configured TaqMan® OpenArray™ Plates* (Pub. No. MAN0017500) for the flexible-content and custom-configured TaqMan® OpenArray™ Plate workflow.

### 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 5–10 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 5–10 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 <sup>[1]</sup>	10 Rxns <sup>[1]</sup>
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
<b>Total Poly(A) Reaction Mix volume</b>	<b>3.0 µL</b>	<b>13.2 µL</b>	<b>33 µL</b>

<sup>[1]</sup> 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 <sup>[1]</sup>	10 Rxns <sup>[1]</sup>
5X DNA Ligase Buffer	3 µL	13.2 µL	33 µL
50% PEG 8000 <sup>[2]</sup>	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
<b>Total Ligation Reaction Mix volume</b>	<b>10 µL</b>	<b>44 µL</b>	<b>110 µL</b>

<sup>[1]</sup> Volumes include 10% overage.

<sup>[2]</sup> 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 <sup>[1]</sup>	10 Rxns <sup>[1]</sup>
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
<b>Total RT Reaction Mix volume</b>	<b>15 µL</b>	<b>66 µL</b>	<b>165 µL</b>

<sup>[1]</sup> 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

- 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 <sup>[1]</sup>	10 Rxns <sup>[1]</sup>
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
<b>Total miR-Amp Reaction Mix volume</b>	<b>45 µL</b>	<b>198 µL</b>	<b>495 µL</b>

<sup>[1]</sup> Volumes include 10% overage.

- Vortex the miR-Amp Reaction Mix, then centrifuge briefly.
- Transfer 45 µL of the miR-Amp Reaction Mix to each well of a *new* reaction plate or reaction tube.
- 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.
- Seal the reaction plate or tubes, then vortex briefly to thoroughly mix the contents.
- Centrifuge the reaction plate or tubes briefly to spin down the contents.
- 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	18–22
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.

## Prepare and run TaqMan® Advanced miRNA Assays with fixed-content OpenArray™ plates

The workflow for fixed-content OpenArray™ plates uses QuantStudio™ 12K Flex Software to generate the plate layouts.

### Generate OpenArray™ plate layouts in the QuantStudio™ 12K Flex Software

Download the EDT file at [thermofisher.com/OA-platefiles](http://thermofisher.com/OA-platefiles).

- In the menu, click **Open**, then navigate to and select the EDT file.
- In the **Home** screen, in the **Setup** menu, click **Experiment Setup**.
- Enter the **Experiment Name** and the **Barcode**.
- In the **Setup** menu, Click **Define**, then enter the sample names in the **Samples** pane.

The plate layout included in the EDT file is designed for one sample (three technical replicates) or three samples (three biological replicates) per reaction.

- Click **File** ▶ **Save as** to save the assay information as an EDS file.

### Set up the real-time PCR reactions in an OpenArray™ 384-well Sample Plate

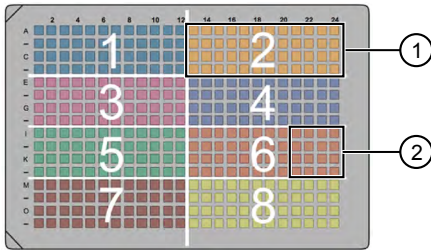
**IMPORTANT!** The 4 × 12 areas of the 384-well plate being filled must match the area designated in the QuantStudio™ 12K Flex Software for that set of samples.

- Remove the OpenArray™ plate from the freezer; allow it to come to room temperature in its unopened sleeve (~15 minutes).  
The OpenArray™ plate must be completely thawed before transferring reactions to it from the 384-well sample plate.
- Prepare a 1:20 dilution of the cDNA template (the miR-Amp reaction product) in 0.1X TE buffer.
- Gently swirl the contents of the TaqMan® OpenArray™ Real-Time PCR Master Mix to thoroughly mix. Do not invert the bottle.
- Combine master mix and cDNA samples in tubes, strip tubes, or a 96-well plate.

Component	Volume per well (OpenArray™ 384-well Sample Plate)	Volume per sample (16 subarrays) <sup>[1]</sup>
TaqMan® OpenArray™ Real-Time PCR Master Mix	2.5 µL	50 µL
Diluted cDNA template	2.5 µL	50 µL
<b>Total reaction volume</b>	<b>5.0 µL</b>	<b>100 µL</b>

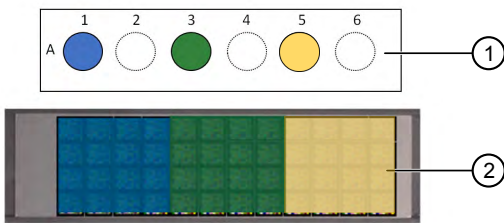
<sup>[1]</sup> Volumes include 25% overage.

- Following the plate layout designated in the EDS file created in the QuantStudio™ 12K Flex Software, add 5.0 µL of the combined master mix and cDNA sample to each well in the sets of 4 × 4 wells in an OpenArray™ 384-well Sample Plate.



- One full array
- One 4 × 4 set of an array (16 subarrays, 1 sample)

If strip tubes or plates are used, load alternate wells to correspond to a 4 × 4 set of the array.



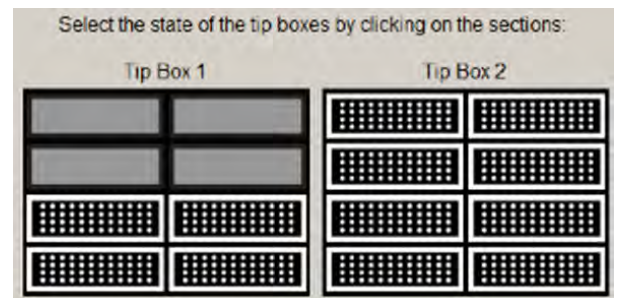
- Samples added to alternating tubes or wells
- 4 × 4 sets of an array

- Thoroughly mix each PCR reaction by pipetting up and down or by using the "mix" function on a multi-channel pipette.
- Seal the plate with an aluminum foil seal, remove the foil flap, then mark the edges of the filled 4 × 12 area with a pen.
- Centrifuge the plate at 1,000 rpm for 1 minute.
- Score the foil along the lines that were marked before centrifuging.  
Do not remove the foil from the scored area at this time.

## Set up the AccuFill™ instrument

**IMPORTANT!** Do not use OpenArray™ AccuFill™ System Tips that exceed the expiration date (shown on the outer box that contains the tip trays).

- In the OpenArray™ AccuFill™ software, click **Setup and Load**.
- In the **Setup Load Information** window, enter the OpenArray™ plate barcodes in the appropriate plate holder positions.  
**Note:** Ensure that the **Use Sample Integration** checkbox is deselected.
- Click the corresponding 4 × 12 area of the 384-well plate, then click **Next** to open the **Setup Deck** window.
- Ensure that:
  - Tip boxes are loaded in the AccuFill™ instrument as illustrated in the following figure:



- Lids are removed from the tip boxes.
- The waste bin in the instrument is emptied.

- In the **Setup Deck** window, select:
  - The tips are configured as shown above**
  - The Waste Bin is empty**

## Transfer reactions to the OpenArray™ plate (AccuFill™ instrument)

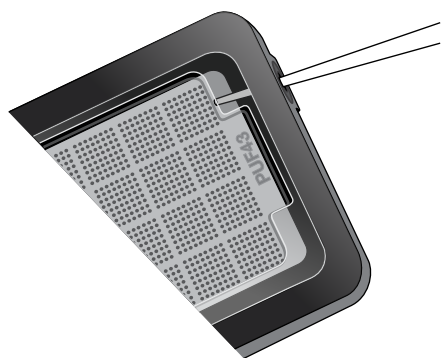
- Prepare the items needed to seal the OpenArray™ plate.  
**Note:** The OpenArray™ plate must be sealed promptly after being loaded with the reactions (this section).
  - Ensure that the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0 is ready.
  - Gather and remove from packaging an OpenArray™ lid, plug, syringe with OpenArray™ Immersion Fluid, and syringe tip.
  - Attach the syringe tip to the syringe and carefully push some of the fluid through the tip to remove air bubbles, then lay the syringe aside.
- Remove the OpenArray™ plate from its sleeve and place it in the plate holder of the AccuFill™ instrument.  
Ensure that the bar code on the OpenArray™ plate is facing left and the serial number is facing right.
- Using forceps, peel the foil from the filled area of the OpenArray™ 384-well Sample Plate.
- Close the instrument door.
- In the AccuFill™ software **Setup Deck** window, select the following confirmations, then click **Load**.
  - The OpenArray Plate is in the Plate Holder**
  - Remove foil from the highlighted section of the Sample Plate**
- As soon as the **Remove OpenArray Plate** window appears, open the instrument door, then remove the loaded OpenArray™ plate.
- Proceed immediately to seal the OpenArray™ plate.  
**Note:** For best results, seal the OpenArray™ plate within 90 seconds of completion of loading, to prevent evaporation.

## Seal the OpenArray™ plate

**IMPORTANT!** Handle the OpenArray™ plate and case only by the edges throughout this procedure.

1. Place the filled OpenArray™ plate in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.  
Ensure that the bar code is facing left and the serial number is facing right.
2. Remove the clear plastic sheet from the inside of the lid, remove the red protective film around the edge of the OpenArray™ lid, then seat the lid on the OpenArray™ case in the plate press.
3. Engage the press mechanism until the green flashing light changes to a steady green light (~20 seconds).
4. Disengage the press, then remove the OpenArray™ case.
5. While holding the OpenArray™ case by the edges, insert the prepared syringe tip into the port in the case, then carefully inject immersion fluid until the case is filled.

**Note:** Minimize creation of air bubbles when you dispense the fluid; one small air bubble in the case is acceptable.



The syringe tip must be in front of the array when filling the case with immersion fluid.




6. While holding the case vertically, remove the syringe tip, insert the screw end of the OpenArray™ plug into the port and rotate clockwise until the black handle breaks off.

**IMPORTANT!** To avoid leaking of immersion fluid, hold the case vertically and rotate the plug slowly.

If the plug handle falls off prematurely, use a Phillips #0 screwdriver to complete the step.


7. Clean the case with a laboratory wipe that has been thoroughly sprayed with ethanol, then dry the case with a clean laboratory wipe.

## Run the OpenArray™ plate(s) on the QuantStudio™ 12K Flex instrument

1. On the instrument touchscreen, touch  to extend the loading arm, then place the OpenArray™ plates on the plate adapter.  
Ensure that the plate barcode and serial number are facing the front of the instrument.
2. Remove the clear plastic sheet from the outside of the plate lid.
3. Touch  to retract the loading arm.
4. In the  **Home** screen of the QuantStudio™ 12K Flex Software, in the **Run** pane, click **OpenArray**.
5. In the **Select Instrument** pane, select your QuantStudio™ instrument.
6. Click **Get Plate IDs** to import the barcodes of the OpenArray™ plates.
7. Click **Import**, then **Browse** to import the EDS file created in “Generate OpenArray™ plate layouts in the QuantStudio™ 12K Flex Software” on page 3.
8. (Optional) Click **Browse** to change the **Experiment File Location**.
9. (Optional) Change the software-determined **Experiment File Name**.
10. Click **Start Run**.  
**Note:** The instrument pauses at 41 or 42 seconds prior to the end of the run. Wait for the system to complete the run before opening the EDS file.
11. Check the QC images for loading issues or leaks.

## Check the QC images

Check the QC images before analysis. For additional information, see *TaqMan® Advanced miRNA Assays User Guide—TaqMan® OpenArray™ Plates* (Pub. No. MAN0016124).

1. In the QuantStudio™ 12K Flex Software  **Export** screen:
  - a. Click **Browse** to create a uniquely-named folder for the QC images export.
  - b. Click **Export QC Images** (bottom of screen).

**IMPORTANT!** Create a new folder for images each time; exporting a second run to the same folder overwrites the images.

2. View the following ROX™ image to check for loading quality issues:
  - POST-READ\_CHANNEL\_4.tiff
3. Check for leaks or other displaced sample issues.
  - a. View the following spotfinding images:
    - s02\_c001\_t03\_p0001\_m1\_x2\_e1\_cp#\_spotfind.tiff


- s02\_c040\_t03\_p0001\_m1\_x2\_e1\_cp#\_spotfind.tiff

**Note:** The "cp#" in the image file name refers to the array position (1–4) within the instrument.

- If a problem is found, view the following pre-run spotfinding image to determine if the issue existed even before cycling (this is useful for troubleshooting):
  - s00\_c001\_t01\_p0001\_m2\_x3\_e1\_cp#\_spotfind.tiff
- View the following FAM™ images to check for any fluorescent abnormalities and to confirm any problem seen in the spotfinding images:
  - STAGE2\_CYCLE1\_CHANNEL\_1.tiff
  - STAGE2\_CYCLE40\_CHANNEL\_1.tiff
- Note any abnormalities found, as well as all other potentially relevant information related to the setup of the run.

## Export and review TaqMan® Advanced miRNA Assay data

### Export data

- Open an EDS file in the QuantStudio™ 12K Flex Software.
- In the **Experiment Menu** pane, click  **Export**.
- Click **Load Export Set** (bottom of the screen), select **GE\_export\_setting**, then click **OK**.
- Select **.xlsx** from the **File Type** dropdown list (top-right of the screen).
- (Optional) Perform any of the following actions to customize the file export:
  - Select **Open file(s) when export is complete**.
  - Click **Browse** to select a new **Export File Location**.
  - Enter a new file name in the **Export File Name** text field.
  - Click the **Results** tab, then select the content to export.
- Click **Start Export** (bottom of the screen).

If **Open file(s) when export is complete** is selected, then the file automatically opens. If the option is not selected, navigate to and open the exported XLSX file.

### Prepare exported data for analysis

- Open the exported XLSX data file.
- Ensure that the barcode, run conditions, and all selected data columns were exported correctly.
- Scroll down to the data rows, select the headers and data, then copy-paste into a new worksheet.
- Rename the new worksheet **Data Table\_Run File Name**.
- (Optional) To combine data from multiple OpenArray™ plates:
  - Insert a **Barcode** column in the **Data Table** worksheet to track OpenArray™ barcodes.

- Copy-paste the barcode numbers to the appropriate cells in the new **Barcode** column.

- Find-replace all "**Undetermined**" values with an empty cell (no value) in the **C<sub>rt</sub>** column.  
This step ensures an exact count of C<sub>rt</sub> values.
- Delete rows that do not contain run data.

### Review results

**Note:** These guidelines apply to results from experiments that included three or more technical replicates.

**Note:** We encourage testing and establishing your own C<sub>rt</sub> cut-off value for each assay to achieve high sensitivity and specificity.

- Review the exported data for through-hole results that may require special attention.
- Consider filtering out from analysis through-holes with the following values:

Parameter to examine	Consider filtering out through-holes if...
C <sub>rt</sub>	C <sub>rt</sub> >28
Amp Score	Amp Score <1.24

**Note:** Through-holes with unexpected C<sub>rt</sub> values can also be identified by reviewing the Amplification Plot.

- Review through-holes with C<sub>rt</sub> >28 and ensure that the C<sub>rt</sub> values are reproducible in all technical replicates.  
**Note:** C<sub>rt</sub>=28 is approximately equal to 1 copy of the target sequence in a reaction.
- Take note of technical replicates with mean C<sub>rt</sub> <28 and a high standard deviation (>0.5). The data from these through-holes may require further review.
- Ensure that at least two of the three replicates amplified adequately and pass your review specifications.
- Use your preferred method to analyze the data.



## Fields for Pivot Tables

To review results using pivot tables, you can use the following settings.

**Note:** For the "Average of" and "StdDev of" summarizations, use the appropriate source field ( $C_{rt}$  or **Amp Score**), then choose the calculation type.

Area of pivot table	Fields to add	
	Target-oriented view	Sample-oriented view
Report Filter	—	Sample Name <sup>[1]</sup>
Column Labels	Sample Name	—

Area of pivot table	Fields to add	
	Target-oriented view	Sample-oriented view
Row Labels	Target Name	Target Name
Values	Average of $C_{rt}$	Average of $C_{rt}$
	StdDev of $C_{rt}$ <sup>[2]</sup>	StdDev of $C_{rt}$
	Count of $C_{rt}$	Count of $C_{rt}$
	—	Average of Amp Score

<sup>[1]</sup> To see individual sample results, select the sample from the dropdown list next to the **Sample Name** header.

<sup>[2]</sup> A **Values** field will automatically appear in the **Column Labels** area.



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**Revision history:** Pub. No. MAN0016125

Revision	Date	Description
B.0	8 January 2018	<ul style="list-style-type: none"> <li>Updated document title.</li> <li>Added reference to Quick Reference for flexible-content and custom-configured TaqMan® OpenArray™ Plates.</li> <li>Corrected plate layout image.</li> <li>Updated TaqMan® OpenArray™ Plate plate handling.</li> <li>Provided troubleshooting for premature break of plug handle.</li> <li>Updated instructions to import files to run the plate on the QuantStudio™ 12K Flex instrument.</li> </ul>
A.0	5 October 2017	New document.

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