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TaqMan® OpenArray® MicroRNA Panels

For microRNA expression analysis on the OpenArray® Real-Time PCR System

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Note: For safety and biohazard guidelines, refer to the "Safety"section in the *TaqMan® OpenArray® MicroRNA Panels User Guide* (Part no. 4461306). For every chemical, read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference is intended for users experienced with TaqMan® OpenArray® MicroRNA Panels and the OpenArray® Real-Time PCR System. Refer to the *TaqMan® OpenArray® MicroRNA Panels User Guide* for background information and detailed instructions.

Input RNA requirements

Use 50–200 ng (100 ng recommended) of total RNA containing the small RNA fraction per Megaplex™ RT reaction. Do not enrich for the small RNA fraction, to avoid loss of longer control transcripts (snoRNAs). Verify the optimal quantity of input total RNA for your sample type.

Reverse transcribe the RNA with Megaplex™ RT Primers

Run two Megaplex™ RT reactions (Pool A and B) per RNA sample.

Set up the RT reactions

1. Thaw the following components on ice, then prepare RT Reaction Mix in each of two 1.5-mL microcentrifuge tubes (one for Pool A, the other for Pool B):

RT Reaction Mix components	Volume per reaction	Volume for 3 reactions†
Megaplex™ RT Primers (10X), Pool A or Pool B‡	0.75 µL	2.5 µL
dNTPs with dTTP (100 mM)	0.15 µL	0.5 µL
MultiScribe Reverse Transcriptase (50 U/µL)	1.50 µL	5.1 µL
10X RT Buffer	0.75 µL	2.5 µL
$MgCl_2$ (25 mM)	0.90 µL	3.0 µL
RNase Inhibitor (20 U/µL)	0.09 µL	0.3 µL
Nuclease-free water	0.35 µL	1.2 µL
Total	4.50 µL	15.1 µL

† Includes 12.5% excess for volume loss from pipetting.
‡ Use Pool A in one tube, and Pool B in the other.

2. Pipet up and down to mix, then centrifuge the tubes briefly.

3. Transfer 4.5 µL of the RT Reaction Mix into the appropriate number of wells of a 96-well MicroAmp® Optical Reaction Plate.

   Note: Each RNA sample is processed in two wells: one for Pool A and one for Pool B. Refer to the plate loading scheme in the *TaqMan® OpenArray® MicroRNA Panels User Guide*.

4. Add 100 ng total RNA in 3 µL to each well containing RT reaction mix.

   Note: Use 3 µL of water for the No Template Control reactions.

5. Mix the reactions in one of these ways:
   - Pipet each mixture up and down a few times, then seal the plate using MicroAmp® Clear Adhesive Film.
   - Seal the plate using MicroAmp® Clear Adhesive Film, then invert the plate 6 times.

   Note: Do not use MicroAmp® *Optical* Adhesive Film to seal the plate.

6. Spin the plate briefly, then incubate the plate on ice for 5 minutes.

Run the RT reactions

1. Set up the run method using the following conditions:
   - Ramp speed or mode: **9700** using **Std** or **Max** ramp speed.
   - Reaction volume (µL): **7.5** (enter 8 µL if your instrument accepts only whole number values)
   - Thermal-cycling conditions:

Stage	Temp	Time
Cycle (40 Cycles)	16°C	2 min
	42°C	1 min
	50°C	1 sec
Hold	85°C	5 min
Hold	4°C	∞

2. Load, then run the plate.

STOPPING POINT (Optional) The RT products (cDNA) can be stored at –15 to –25°C for at least one week.

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Preamplify the cDNA with Megaplex™ PreAmp Primers

Set up the preamplification reactions

1. Thaw the Megaplex™ PreAmp Primers on ice and mix by inverting 6 times. Spin briefly.
2. Swirl the bottle of TaqMan® PreAmp Master Mix (2X) to mix.
3. Prepare PreAmp Reaction Mix for Pool A and Pool B in each of two 1.5-mL microcentrifuge tubes:

PreAmp Reaction Mix components	Volume per reaction	Volume for 3 reactions†
TaqMan® PreAmp Master Mix (2X)	12.5 µL	42.4 µL
Megaplex™ PreAmp Primers (10X), Pool A or Pool B‡	2.5 µL	8.4 µL
Nuclease-free water	7.5 µL	25.3 µL
Total	22.5 µL	76.1 µL

† Includes 12.5% excess for volume loss from pippetting.
‡ Use Pool A in one tube, and Pool B in the other.

4. Pipet up and down to mix, then centrifuge the tubes briefly.
5. Transfer 2.5 µL of each RT product into one well of a clean MicroAmp® Optical 96-well Reaction Plate (two wells per sample, one for Pool A and one for Pool B).
6. Dispense 22.5 µL of PreAmp Reaction Mix Pool A or Pool B into each well containing the corresponding RT product.
7. Mix the reactions in one of these ways:
   - Pipet each mixture up and down a few times, then seal the plate using MicroAmp® Clear Adhesive Film.
   - Seal the plate using MicroAmp® Clear Adhesive Film, then invert the plate 6 times.
8. Spin the plate briefly, then incubate the plate on ice for 5 minutes.

Run the preamplification reaction

Set up the run method using the following conditions:

- Ramp speed or mode: **9700** using **Std** ramp speed.
- Reaction volume (µL): **25**
- Thermal-cycling parameters:

Stage	Temp	Time
Hold	95°C	10 min
Hold	55°C	2 min
Hold	72°C	2 min
Cycle	95°C	15 sec
(12 Cycles)	60°C	4 min
Hold†	99.9°C	10 min
Hold	4°C	∞

† Required for enzyme inactivation.

Dilute the preamplification products

1. Remove the 96-well plate from the thermal cycler, and briefly centrifuge to collect the contents at the bottom of each well.
2. For each preamplification reaction, add 156 µL of 0.1X TE pH 8.0 to one well of a new 96-well plate.
3. Transfer 4 µL of each preamplification reaction to each well containing 0.1X TE buffer.
4. Mix the diluted products in one of these ways:
   - Pipet up and down a few times, then seal the 96-well plate.
   - Seal the 96-well plate, then invert the plate 6 times.
5. Centrifuge the plate briefly, then place the plate on ice.

   Note: The diluted preamplification product is stable for up to 12 hours at 4°C.

STOPPING POINT (Optional) Store the diluted preamplified product (diluted or undiluted) at –15 to –25°C for up to one week.

Run the TaqMan® OpenArray® MicroRNA Panels

Access the setup file(s) and prepare the panels

1. Download the plate setup file (*.tpf) for the TaqMan® OpenArray® MicroRNA Panel from the OpenArray® Plate product page at **www.lifetechnologies.com** (Applied Biosystems).
2. Remove the TaqMan® OpenArray® MicroRNA Panel from the freezer and allow it to come to room temperature (approximately 15 minutes).

Prepare the PCR Reaction Mixes

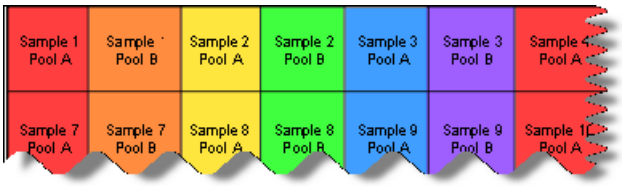
1. If frozen, fully thaw the diluted, stored preamplification products on ice. Mix by inverting the sealed plate 6 times or by vortexing gently, then centrifuge the plate briefly.
2. Mix the TaqMan® OpenArray® Real-Time PCR Master Mix by swirling the bottle.
3. For each sample, transfer 22.5 µL of TaqMan® OpenArray® Real-Time PCR Master Mix into each of two adjacent wells on a clean 96-well plate. See the plate loading scheme in the *TaqMan® OpenArray® MicroRNA Panels User Guide* for detailed layout recommendations.
4. For each sample, pipet 22.5 µL of diluted Pool A preamplification product into one well of each pair, and 22.5 µL of diluted Pool B preamplification product into the other well.
5. Seal the plate, vortex gently to mix, and centrifuge the plate briefly.

Note: The assembled PCR Reaction Mix can be kept at 4°C for up to 12 hours in the 96-well plate before transferring to the 384-well plate.

Load and run the TaqMan® OpenArray® MicroRNA Panel

For detailed information on loading and sealing the TaqMan® OpenArray® MicroRNA Panel using the AccuFill™ System, refer to the *OpenArray® AccuFill™ System User Guide* (Part no. 4456986). For information on running PCR with the TaqMan® OpenArray® MicroRNA Panel, refer to the *OpenArray® Real-Time PCR System User Guide* (Part no. 4458837).

1. Dispense 5 µL of each PCR Reaction Mix into each of 8 wells on an OpenArray® 384-Well Sample Plate, as shown on the plate map below (each square represents eight sample wells). Use a second plate if needed to hold all samples.



2. After the 384-well plate is filled with the PCR Reaction Mixes, and the TaqMan® OpenArray® MicroRNA Panel has reached room temperature, carefully remove it from its packaging.
3. Load the TaqMan® OpenArray® MicroRNA Panel from the 384-well plate, using the standard AccuFill™ method.
4. Run the TaqMan® OpenArray® MicroRNA Panel using the .tpf file corresponding to the serial number for each plate.

Note: The TaqMan® OpenArray® MicroRNA Panel must be run within one hour of loading.

Analyze the data

Export the data and analyze with DataAssist™ software. For detailed information on how to export and analyze primary real-time data, refer to the *OpenArray® Real-Time PCR System User Guide* (Part no. 4458837).

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