TaqMan™ Advanced miRNA Assays

USER GUIDE

TaqMan™ Array Cards

for use with:

TaqMan™ Advanced miRNA cDNA Synthesis Kit

Publication Number MAN0016122

Revision D.0

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ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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Revision	Date	Description
D.0	31 August 2021	• Added the QuantStudio™ 7 Pro Real-Time PCR System and the QuantStudio™ Design and Analysis Software v2. • Updated centrifuges. • Updated the name of the cloud-based platform to Thermo Fisher™ Connect and updated the URLs. • Provided calculations for 8 reactions (a full TaqMan™ Array Card) instead of 10 reactions to prepare the cDNA templates. • Specified that the volume of water should be reduced to compensate for the optional RNase Inhibitor when performing the poly(A) tailing reaction. • Added recommendations for algorithms and data analysis. • Added guidelines for storage if the TaqMan™ Array Card is not run immediately. • Specified that a centrifuge speed that is too high can deform the TaqMan™ Array Card (Appendix B, “Detailed procedures for preparation of a TaqMan™ Array Card”). • Added documents for the QuantStudio™ 7 Flex Real-Time PCR System (Appendix E, “Documentation and support”).
C.0	3 January 2018	• Updated product description and available formats to include flexible-content and custom-configured assays. • Added descriptions of relevant Thermo Fisher™ Connect applications. • Updated the recommended data analysis method for 7900HT Fast Real-Time PCR System.
B.0	21 November 2017	• Updated list of recommended RNA isolation kits and thermal cyclers. • Updated instructions for mixing TaqMan™ Fast Advanced Master Mix. • Updated list and description of files included with the assays and updated instructions for the file types. • Provided instructions to import files into real-time PCR instrument or software and specified which files correspond with each instrument. • 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.

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1 Product information

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Product description

TaqMan™ Advanced miRNA Assays are pre-formulated primer and probe sets that are designed for analysis of microRNA (miRNA) expression levels using Applied Biosystems™ real-time PCR instruments. The assays can detect and quantify the mature form of the miRNA from:

- 1–10 ng of total RNA from tissue.
- 2 µL of sample eluent from total RNA isolation from serum or plasma.

For more information about PCR detection with TaqMan™ Advanced miRNA Assays, see “Overview of TaqMan™ Advanced miRNA Assays chemistry” on page 37.

The TaqMan™ Advanced miRNA cDNA Synthesis Kit (Cat. No. A28007; sold separately) is required for preparing the cDNA template that is used with the TaqMan™ Advanced miRNA Assays. The kit enables the analysis of:

- Multiple miRNAs from a single amplified sample.
- Samples that are limited in quantity, including serum, plasma, or other biological fluids.

The procedures in this document are for use with TaqMan™ Advanced miRNA Assays in the configurations in the following table:

Configuration	Description	Customizable
Fixed-content TaqMan™ Array Cards	Preplated and predefined TaqMan™ Advanced miRNA Assays that are manufactured and stocked in advance	No
Flexible-content TaqMan™ Array Cards	TaqMan™ Array Cards configured with a suggested selection of TaqMan™ Advanced miRNA Assays, categorized by specific disease, pathway, or biological process	Preselected assays can be substituted with other predesigned assays that target TaqMan™ Advanced miRNA Assays more applicable to your experiment needs
Custom-configured TaqMan™ Array Cards	Fully customizable TaqMan™ Array Cards	Allows the configuration of the TaqMan™ Array Cards with any predesigned assays

This document describes procedures to prepare cDNA templates from miRNA followed by PCR amplification of the cDNA template and subsequent data analysis.

First, mature miRNAs from total RNA are modified by extending the 3' end of the mature transcript through poly(A) addition, then lengthening the 5’ end by adaptor ligation. The modified miRNAs then undergo universal reverse transcription followed by amplification to increase uniformly the amount of cDNA for all miRNAs (miR-Amp reaction). For more information about cDNA synthesis of templates for TaqMan™ Advanced miRNA Assays, see “Overview of cDNA template preparation” on page 36.

The cDNA templates are then used with TaqMan™ Advanced miRNA Assays for quantification of miRNA expression levels by qPCR analysis. Predesigned TaqMan™ Advanced miRNA Assays are available for most human miRNAs in miRBase (the miRNA sequence repository). For a current list of assays, go to **thermofisher.com/advancedmirna**.

Note: TaqMan™ Advanced miRNA Assays are for analysis of mature miRNA only. For analysis of siRNA, or other small RNAs that are fewer than 200 bases in length, go to **thermofisher.com/taqmanmirna**.

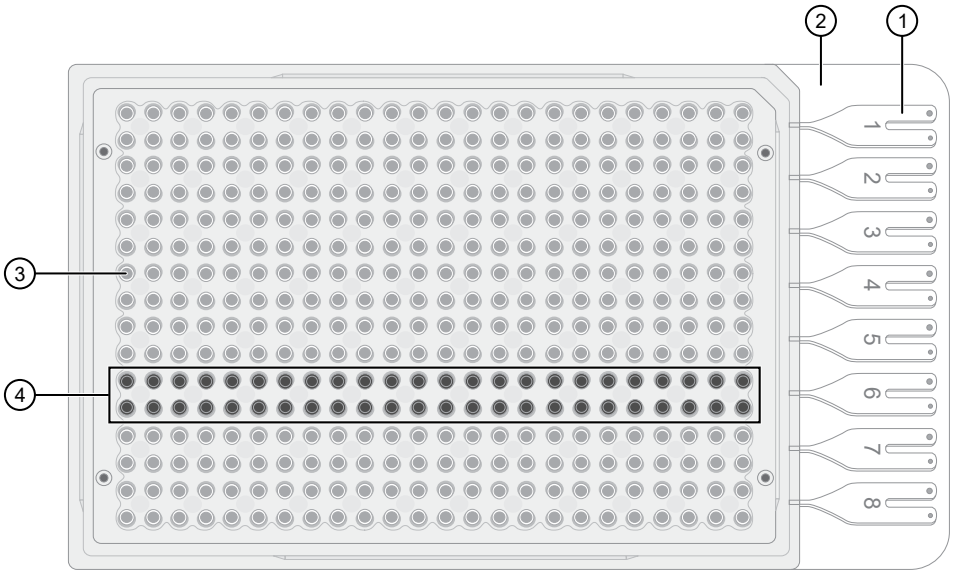
Overview of a TaqMan™ Array Card

A TaqMan™ Array Card is a 384-well microfluidic card that is prepared with dried-down TaqMan™ Assays. With an array card, gene expression is measured using the comparative C_t ($\Delta\Delta C_t$) method of relative quantitation.

Advantages of a TaqMan™ Array Card include the following:

- Small-volume design that minimizes sample and reagent consumption.
- Streamlined reaction setup that saves time and reduces labor-intensive steps.
- Access to high-throughput, 384-well format without liquid-handling robotics.
- Two-fold discrimination detection at the 99.7% confidence level.
- Standardization across multiple samples in multiple laboratories.

Each card can run 1 to 8 samples against 12 to 384 TaqMan™ Assay targets (including controls).



① **Fill reservoir**—Each reservoir is loaded with a sample-specific PCR reaction mix; the associated reaction wells fill with that sample (8 total reservoirs)

② **Fill reservoir strip**—Support strip for fill reservoirs; removed before running the card

③ **Reaction well**—Each well contains dried-down assay (384 total reaction wells)

④ **Reaction well row**—A set of reaction wells that fill with the same sample-specific PCR reaction mix (8 total rows, each row associated with a single fill reservoir)

Contents and storage

Table 1 TaqMan™ Advanced miRNA Assays (with a fixed-content TaqMan™ Array Card)

Panel	Cat. No.	Amount	Storage[1]
TaqMan™ Advanced miRNA Human A and B Cards	A31805	2 cards	2–8°C[2]
TaqMan™ Advanced miRNA Human A Card	A31806	1 card	
TaqMan™ Advanced miRNA Human B Card	A31807	1 card	
TaqMan™ Advanced miRNA Human Serum/Plasma Card	A31808	1 card	
TaqMan™ Advanced miRNA Human Endogenous Controls Card	A31809	1 card	

[1] See packaging for expiration date.

[2] Shipped at ambient temperature. See thermofisher.com/ambientshippping.

Table 2 TaqMan™ Advanced miRNA Assays (with a flexible-content or a custom-configured TaqMan™ Array Card)

Format	Cat. No.	Number of assays + manufacturing control[1]	Number of samples	Storage[2]
TaqMan™ Array Card 12	A34718	11+ 1	8 quadruplicates	2–8°C[3]
TaqMan™ Array Card 16	A34719	15+ 1	8 triplicates	
TaqMan™ Array Card 24	A34720	23+ 1	8 duplicates	
TaqMan™ Array Card 32	A34721	31+ 1	4 triplicates	
TaqMan™ Array Card 48	A34722	47+ 1	8 no replicates	
TaqMan™ Array Card 64	A34723	63+ 1	2 triplicates	
TaqMan™ Array Card 96a	A34724	95+ 1	4 no replicates	
TaqMan™ Array Card 96b	A34725	95+ 1	2 duplicates	
TaqMan™ Array Card 192	A34726	191+ 1	1 duplicates	
TaqMan™ Array Card 384	A34727	380+ 4	1 no replicates	

[1] hsa-miR-16-5p, Assay ID: 477860_mir

[2] See packaging for expiration date.

[3] Shipped at ambient temperature. See thermofisher.com/ambientshippping.

To order a flexible-content TaqMan™ Array Card, go to **thermofisher.com/flexiblepanels**.

To order a custom-configured TaqMan™ Array Card, go to **thermofisher.com/arraycards**.

Go to **thermofisher.com/taqmanfiles** to download the following files:

- **Plate layout files (HTML and CSV formats)**—Show the position of each assay on the card. The HTML and CSV files contain identical information.
- **Setup files (SDS in TXT format)**—Contain the sample setup on the card. The files are imported into the software specific to your instrument to perform real-time PCR.
- **Assay Information File (AIF in TXT format)**—Describes the TaqMan™ Advanced miRNA Assays. See *Understanding Your Shipment* (Pub. No. MAN0017153) for detailed information about the AIF.

Note: During the download, you may be asked to enter specific order numbers or product information. The run properties and the thermal protocol are not defined in the setup files and must be set up in the instrument or software.

Required materials

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Table 3 Recommended RNA isolation kits

Sample type	Kit	Cat. No.
Tissue samples	*mir*Vana™ miRNA Isolation Kit, with phenol	AM1560
	*mir*Vana™ miRNA Isolation Kit, without phenol	AM1561
	MagMAX™ *mir*Vana™ Total RNA Isolation Kit	A27828
	*mir*Vana™ PARIS™ RNA and Native Protein Purification Kit	AM1556
Serum / Plasma samples	Total Exosome RNA and Protein Isolation Kit	4478545
	MagMAX™ *mir*Vana™ Total RNA Isolation Kit	A27828
	*mir*Vana™ PARIS™ RNA and Native Protein Purification Kit	AM1556
Cell samples	TaqMan™ MicroRNA Cells-to-C_T Kit	4391848
FFPE samples	RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	AM1975

Table 4 TaqMan™ Advanced miRNA cDNA Synthesis Kit (Cat. No. A28007, 50 reactions)

Contents	Storage
10X Poly(A) Buffer	–20°C
ATP, 10 mM	
Poly(A) Enzyme, 5 U/µL	
5X DNA Ligase Buffer	
RNA Ligase, 10 U/µL	
50% PEG 8000	
25X Ligation Adaptor	
10X RT Enzyme Mix	
5X RT Buffer	
20X Universal RT Primer	
dNTP Mix, 100 mM	
20X miR-Amp Primer Mix	
2X miR-Amp Master Mix	2–4°C

Table 5 Other materials and equipment required for the workflow

Item	Source
Real-time PCR instrument, one of the following:	
The instrument must be configured with the TaqMan™ Array Card block and heated cover.	
QuantStudio™ 7 Pro Real-Time PCR System	Contact your local sales office
QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 12K Flex Real–Time PCR System	
ViiA™ 7 Real-Time PCR System	
7900HT Fast Real-Time PCR System	
Software	
QuantStudio™ Design and Analysis Software v2 For use with the QuantStudio™ 7 Pro Real-Time PCR System. Can be used to analyze data files from all of the other compatible instruments.	**thermofisher.com/us/en/home/global/forms/life-science/quantstudio-6-7-pro-software**
(Optional) Relative Quantification application	Available on Thermo Fisher™ Connect
(Optional, for E1–ClipTip™ Pipettes) My Pipette™ Creator app	Available on Thermo Fisher™ Connect
Equipment	
Thermal cycler, one of the following (or equivalent): • Veriti™ Thermal Cycler • SimpliAmp™ Thermal Cycler • ProFlex™ PCR System	Contact your local sales office
Centrifuge with custom buckets and card holders, one of the following: • Sorvall™ centrifuge • Megafuge™ centrifuge • Multifuge™ centrifuge See the **Resources** section at **thermofisher.com/taqmanarrays** for a list of compatible centrifuges, rotors, and buckets.	Contact your local sales office
TaqMan™ Array Card Sealer (Referred to as Stylus Staker in some documents)	Contact your local sales office
Blank balance TaqMan™ Array Cards (Included with the instrument block upgrade / installation kit)	Contact your local sales office
Microcentrifuge	MLS

Item	Source
Vortex mixer	MLS
(Optional) Eppendorf™ MixMate™ (shaker)	Fisher-Scientific 21-379-00
Pipettes	MLS
(Optional) E1–ClipTip™ Pipette	Contact your local sales office
Micropipettes	MLS
Tubes, plates, and other consumables	
Plastics consumables	**thermofisher.com/plastics**
Aerosol-resistant barrier pipette tips	MLS
Disposable gloves	MLS
Reagents	
Nuclease-free water	MLS
Tris-EDTA (TE) Buffer, pH 8.0	MLS
TaqMan™ Fast Advanced Master Mix	4444557

Endogenous and exogenous controls

For information about using endogenous or exogenous controls with TaqMan™ Advanced miRNA Assays, see “Endogenous and exogenous controls” on page 34.

See *A technical guide to identifying miRNA normalizers using TaqMan™ Advanced miRNA Assays White Paper* (Pub. No. COL31302 0916) for available TaqMan™ Advanced miRNA Assays that target miRNAs with relatively constant expression levels across many different sample types (available at **thermofisher.com/advancedmirna**, in the **Resources** section).

Table 6 Endogenous control assay

Assay name[1]	Assay ID	Target Sequence
hsa-miR-16-5p	477860_mir	5′-UAGCAGCACGUAAAUAUUGGCG-3′

[1] TaqMan™ Advanced miRNA Assays do not detect snRNAs or snoRNAs. Do not use snRNAs and snoRNAs as endogenous controls for these assays.

Table 7 Exogenous control assays (for human samples)

Assay Name	Assay ID	Target Sequence[1]
ath-miR159a[2]	478411_mir	5′-UUUGGAUUGAAGGGAGCUCUA-3′
cel-miR-39-3p[3]	478293_mir	5′-UCACCGGGUGUAAAUCAGCUUG-3′

[1] Oligonucleotides for exogenous controls must be 5'-phosphorylated.
[2] From *A. thaliana.*
[3] From *C. elegans.*

Thermo Fisher™ Connect applications

Application	Description
Relative Quantification 	• Allows analysis of data with the relative threshold (C_{rt}) algorithm. – C_{rt} is an alternative to the baseline threshold algorithm (C_t). – C_{rt} can be used if the baseline is variable. This variability might be caused by reconstitution of dried-down assays on the card at different rates. C_{rt} can correct for a variable baseline. • The application can be used for data analysis for convenience or if the software specific to your instrument does not have the relative threshold algorithm.
My Pipette™ Creator 	• Allows the creation of assay-specific pipetting programs for the E1–ClipTip™ Pipette. • Includes pre-written and validated protocols for Thermo Fisher Scientific assays, including TaqMan™ Advanced miRNA Assays.

Workflow

Prepare cDNA templates

Input RNA sample

▼

Perform the poly(A) tailing reaction (page 15)

(55 minutes)

▼

Perform the adaptor ligation reaction (page 16)

(60 minutes)

▼

Perform the reverse transcription (RT) reaction (page 17)

(20 minutes)

▼

Perform the miR-Amp reaction (page 18)

(30 minutes)

▼

Perform real-time PCR

Prepare PCR reactions (page 20)

▼

Load the PCR reaction mix (page 29),

Centrifuge the card (page 29), and

Seal the card (page 31)

▼

Set up and run the real-time PCR instrument (page 21)

(45 minutes)

▼

Analyze the results (page 22)

2 Prepare cDNA templates

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- Perform the poly(A) tailing reaction 15
- Perform the adaptor ligation reaction 16
- Perform the reverse transcription (RT) reaction 17
- Perform the miR-Amp reaction 18

Procedural guidelines

Guidelines for RNA input

- Prepare samples using a total RNA isolation method that preserves small RNAs.
  See Table 3 on page 9 for recommended RNA isolation kits.
- For tissue samples, use 1–10 ng of total RNA per reaction.

  Note: Sample concentration before adding to reactions should be ≤5 ng/µL.
- 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 (not applicable for double-stranded templates)

    IMPORTANT! Do not denature the total RNA.

Guidelines for preparing cDNA templates

- Follow best practices when working with RNA samples (see “Best practices for PCR and RT-PCR experiments” on page 40).
- Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes, then include 10% overage.
- If using strip tubes, change to a new strip cap after each step or incubation.
- 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 to thoroughly mix, then centrifuge briefly to collect the contents at the bottom of the tube and eliminate air bubbles.

   Note: Do not remove the TaqMan™ Array Cards from storage until you are ready to perform real-time PCR.

   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 reaction	4 reactions[1]	8 reactions[1]
10X Poly(A) Buffer	0.5 µL	2.2 µL	4.4 µL
ATP	0.5 µL	2.2 µL	4.4 µL
Poly(A) Enzyme	0.3 µL	1.3 µL	2.6 µL
RNase–free water	1.7 µL	7.5 µL	15.0 µL
Total Poly(A) Reaction Mix volume	**3.0 µL**	**13.2 µL**	**26.4 µL**

[1] Volumes include 10% overage.

3. Vortex the Poly(A) Reaction Mix to thoroughly mix the contents, then centrifuge briefly to collect the contents at the bottom of the tube and eliminate air bubbles.

4. Add 2 µL of sample to each well of a reaction plate or each reaction tube.

   Note: *(Optional)* Before adding the sample to the reaction plate or tube, add RNase Inhibitor to each sample to minimize the effects of RNase contamination.
   For detailed instructions, see the documentation for the RNase Inhibitor.

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.

   Note: Decrease RNase–free water as required to compensate for RNase Inhibitor.

6. Seal the reaction plate or tubes, then vortex briefly to thoroughly mix the contents.

7. Centrifuge the reaction plate or tubes briefly to collect the contents at the bottom 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 "Perform the adaptor ligation reaction" on page 16.

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 reaction	4 reactions[1]	8 reactions[1]
5X DNA Ligase Buffer	3 µL	13.2 µL	26.4 µL
50% PEG 8000[2]	4.5 µL	19.8 µL	39.6 µL
25X Ligation Adaptor	0.6 µL	2.6 µL	5.3 µL
RNA Ligase	1.5 µL	6.6 µL	13.2 µL
RNase-free water	0.4 µL	1.8 µL	3.5 µL
Total Ligation Reaction Mix volume	**10 µL**	**44 µL**	**88 µL**

[1] Volumes include 10% overage.
[2] 50% PEG 8000 is very viscous. Follow instructions in 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 the solution slowly.
  a. Hold the pipette tip in the solution for approximately 10 seconds after slowly releasing the plunger during aspiration. This action allows the solution to be fully drawn into the pipette tip.
  b. Keep the plunger depressed for approximately 10 seconds to allow the solution to be fully dispensed into the Ligation Reaction Mix.

2. Vortex the Ligation Reaction Mix to thoroughly mix, then centrifuge briefly to collect the contents at the bottom of the tube and eliminate air bubbles.

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™ 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 collect the contents at the bottom.

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 "Perform the reverse transcription (RT) reaction" on page 17.

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 reaction	4 reactions[1]	8 reactions[1]
5X RT Buffer	6 µL	26.4 µL	52.8 µL
dNTP Mix (25 mM each)	1.2 µL	5.3 µL	10.6 µL
20X Universal RT Primer	1.5 µL	6.6 µL	13.2 µL
10X RT Enzyme Mix	3 µL	13.2 µL	26.4 µL
RNase-free water	3.3 µL	14.5 µL	29.0 µL
Total RT Reaction Mix volume	**15 µL**	**66 µL**	**132 µL**

[1] Volumes include 10% overage.

2. Vortex the RT Reaction Mix to thoroughly mix the contents, then centrifuge briefly to collect the contents at the bottom of the tube and eliminate air bubbles.

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 collect the contents at the bottom.

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 "Perform the miR-Amp reaction" on page 18, 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 reaction	4 reactions[1]	8 reactions[1]
2X miR-Amp Master Mix	25 µL	110 µL	220 µL
20X miR-Amp Primer Mix	2.5 µL	11 µL	22 µL
RNase-free water	17.5 µL	77 µL	154 µL
Total miR-Amp Reaction Mix volume	**45 µL**	**198 µL**	**396 µL**

[1] Volumes include 10% overage.

2. Vortex the miR-Amp Reaction Mix to thoroughly mix the contents, then centrifuge briefly to collect the contents at the bottom of the tube and eliminate air bubbles.

3. Transfer 45 µL of the miR-Amp Reaction Mix to each well of a reaction plate or reaction tubes.

IMPORTANT! Use a *new* reaction plate or *new* reaction tubes.

4. Add 5 µL of the RT reaction product to each well or 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 collect the contents at the bottom.

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	Hold

Proceed to Chapter 3, “Perform real-time PCR”, or store the undiluted miR-Amp reaction product at –20°C for up to 2 months.

3 Perform real-time PCR

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- Prepare PCR reactions 20
- Prepare a TaqMan™ Array Card 21
- Set up and run the real-time PCR instrument 21
- Analyze the results 22

Procedural guidelines for performing real-time PCR

- Follow best practices when performing PCR reactions (see “Best practices for PCR and RT-PCR experiments” on page 40).
- For instructions to prepare a TaqMan™ Array Card, see Appendix B, “Detailed procedures for preparation of a TaqMan™ Array Card”.
- Prepare the real-time PCR reactions in an area free of artificial templates and siRNA transfections. High-copy-number templates can easily contaminate the real-time PCR reactions.
- Configure run documents according to the instructions provided in the real-time PCR instrument resource documents.

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 Appendix B, "Detailed procedures for preparation of a TaqMan™ Array Card".

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 software specific to your instrument.
   See page 39 for instructions to import setup files.

   Note: The setup files are instrument-specific. See page 39 for more information.

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.
   The following thermal protocols are optimized for use with TaqMan™ Fast Advanced Master Mix.

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

   [1] To completely dissolve the assay on the card. Ensure that the time is set correctly for cards. All instruments have a default of 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. Use 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.

Instrument	Data analysis settings
QuantStudio™ 7 Pro 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™ 7 Flex Real-Time PCR System	
QuantStudio™ 12K Flex Real–Time PCR System	
ViiA™ 7 Real-Time PCR System	
7900HT Fast Real-Time PCR System	• Use the relative threshold (C_{rt}) algorithm. It is available in the Relative Quantification application, on Thermo Fisher™ Connect.[1] • Alternatively, use the threshold (C_t) method and use an 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.

[1] See "Thermo Fisher™Connect applications" on page 12 for more information.

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 application	**thermofisher.com/us/en/home/digital-science/thermo-fisher-connect.html**
ExpressionSuite™ Software[1]	**thermofisher.com/us/en/home/technical-resources/software-downloads/expressionsuite-software.html**

[1] ExpressionSuite™ Software automatically defines the optimal threshold value.

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

Algorithms for data analysis

Table 8 Algorithm recommendations for TaqMan™ Array Cards

Algorithm	Recommendation
Relative threshold (C_{rt})	Recommended for the following instruments: • QuantStudio™ Real-Time PCR Instruments • ViiA™ 7 instrument Can correct a variable baseline, which might be due to dried-down assays on the card being reconstituted at different rates.
Threshold (C_t)	Optional if used for analysis of established protocols. Recommended for 7900HT Fast Real-Time PCR Instrument.

The relative threshold algorithm is available in the Relative Quantification application on Thermo Fisher™ Connect (**thermofisher.com/connect**).

A

Troubleshooting

- Troubleshooting: After removing the card from packaging 24
- Troubleshooting: After loading PCR reaction mix into the card 24
- Troubleshooting: After centrifuging the card 25
- Troubleshooting: After running the card and reviewing run results 25

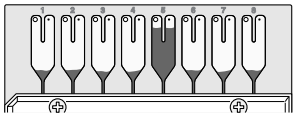
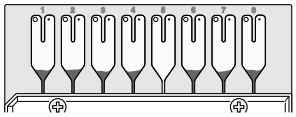
Troubleshooting: After removing the card from packaging

Observation	Possible cause	Recommended action
Water condenses on the reaction wells (optical side of the card)	The card was not at room temperature before being removed from the packaging.	Remove condensation on the reaction wells by lightly blowing room temperature pressurized nitrogen or an air blower on the wells. **IMPORTANT!** Ensure that all water condensation is removed. The optical side of the card must be free of water condensation.

Troubleshooting: After loading PCR reaction mix into the card

Observation	Possible cause	Recommended action
Fill reservoir is not full of PCR reaction mix	The PCR reaction mixture was not correctly pipetted into the fill reservoir.	Be sure to correctly pipette the entire PCR reaction mixture (100 µL) into the fill reservoir. Add more sample-specific PCR reaction mix to the fill reservoir.
PCR reaction mix leaks from the vent port into the fill reservoir	The PCR reaction mixture was not correctly pipetted into the fill reservoir.	Be sure to correctly pipette the entire PCR reaction mixture (100 µL) into the fill reservoir. Add more sample-specific PCR reaction mix to the fill reservoir.
Fill reservoirs have bubbles in the PCR reaction mix	When loading the card with PCR reaction mix, air was introduced into the fill reservoir.	Inspect the affected rows after centrifuging and sealing the card. Note wells that contain bubbles, then consider omitting these wells from analysis.

Troubleshooting: After centrifuging the card

Observation	Possible cause	Recommended action
PCR reaction mix remains in a fill reservoir 	Though rare, the fill port is blocked.	Inspect the card for blocked fill port or a pinched channel. If the fill reservoir is defective, contact Support.
	Filling is incomplete or not consistent.	Centrifuge the card again for 1 minute.
		If the filling is still incomplete after the additional centrifuge cycle, continue with running the card. However, consider omitting the wells associated with that fill reservoir.
Fill reservoir completely empty 	Some wells were filled improperly.	Continue with running the card. However, consider omitting the wells associated with that fill reservoir.

Troubleshooting: After running the card and reviewing run results

Observation	Possible cause	Recommended action
Noise in the amplification plots for portions of the card	The card was misaligned in the block during the instrument run.	Inspect the card for crushed or distorted feet. If there are damaged feet, contact Support.  ① Array card feet
No amplification for portions of the card	The card was misaligned in the block during the instrument run.	Inspect the card for crushed or distorted feet. If there are damaged feet, contact Support.  ① Array card feet
No amplification in some wells	Empty wells due to improper card sealing.	When sealing the card, use a slow and steady motion to push the carriage across the TaqMan™ Array Card Sealer. **IMPORTANT!** Do not move the carriage back across the card. See “Seal the card” on page 31.

Observation	Possible cause	Recommended action
No amplification within or across one or more rows	Empty wells due to improper card sealing.	When sealing the card, use a slow and steady motion to push the carriage across the TaqMan™ Array Card Sealer.
	Empty wells due to misalignment of the TaqMan™ Array Card Sealer.	If the TaqMan™ Array Card Sealer is misaligned, contact Support.
	PCR reaction mix improperly prepared.	Ensure that all reaction components were added to the PCR reaction mix.
Replicates have poor precision **Details:** Poor precision is when the standard deviation value is >0.5 for assays with a C_t value <30.	Bubbles in wells.	Use proper pipetting techniques to avoid introducing air into the fill reservoirs. Consider omitting the leaking wells from analysis.
	Wells leaking due to improper card sealing.	When sealing the card, use a slow and steady motion to push the carriage across the TaqMan™ Array Card Sealer. Consider omitting the leaking wells from analysis. **IMPORTANT!** Do not move the carriage back across the card. See "Seal the card" on page 31.
	Not all of the positions in the card holder were filled before centrifuging.	Ensure that all of the empty positions of the card holder are filled with blank balance cards before centrifuging.
	The cards were centrifuged using a non-verified centrifuge.	Use a verified centrifuge (see "Required materials" on page 9). See the **Resources** section at **thermofisher.com/taqmanarrays** for a list of compatible centrifuges.
	The dried-down assays on the card were reconstituted at different rates, causing a dip in the early cycles of the baseline.	Analyze results with the relative threshold algorithm (C_{rt}) instead of the baseline threshold algorithm (C_t).
The baseline is variable	The dried-down assays on the card were reconstituted at different rates, causing a dip in the early cycles of the baseline.	Use the relative threshold algorithm (C_{rt}). C_{rt} can correct for a variable baseline.
		Use the Relative Quantification application, available on Thermo Fisher™ Connect. The Relative Quantification application uses C_{rt} if the software specific to your instrument does not have the relative threshold algorithm.
No miRNA is detected	The threshold is set too high to detect miRNA in samples with low expression.	Identify an appropriate NOAMP flag threshold if the relative threshold algorithm (C_{rt}) is used.
		Identify an appropriate threshold if C_t is used.
Serum or plasma samples have a high C_t value	The amount of amplified cDNA is low.	Increase the miR-Amp reaction product by increasing the number of cycles from 14 to 18.

B Detailed procedures for preparation of a TaqMan™ Array Card

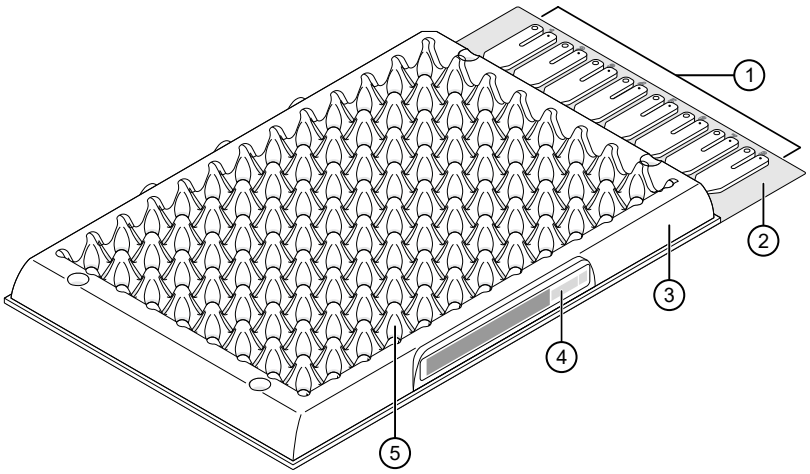
Guidelines for preparation of a card

- Keep the card protected from light and stored as indicated until ready for use. Excessive exposure to light may affect the fluorescent probes of the dried-down assays in the card.
- Before removing the card from its packaging:
  - Prepare each sample-specific PCR reaction mix.
  - Allow the card to reach room temperature.
- Load each fill reservoir with 100 µL of sample-specific PCR reaction mix.
  - Each fill reservoir contains a single sample as determined by the card layout.
  - The 100–µL volume ensures adequate filling of each reaction well. Volumes smaller than 100 µL result in insufficiently filled cards.
- Do not allow the micropipette tip to contact the coated foil beneath the fill port.

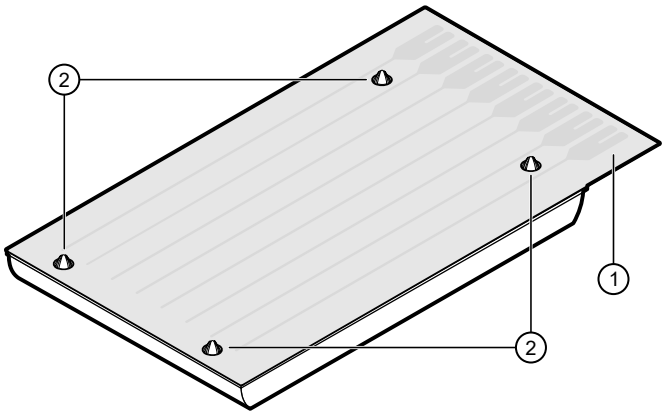
- Load the card with PCR reaction mix *before* centrifuging the card.
  During centrifugation, the PCR reaction mix resuspends the dried-down assays in each well of the card. Adding sample after centrifuging disrupts the assay layout of the card.
- Run the card within 72 hours of sealing the card. Protect the card from light and store at 2–8°C. After 72 hours, well-to-well mixing might occur.
- If the card is not run immediately, protect it from light and store at 2-8°C.

TaqMan™ Array Card diagram

A TaqMan™ Array Card includes 8 fill reservoirs and 384 reaction wells.

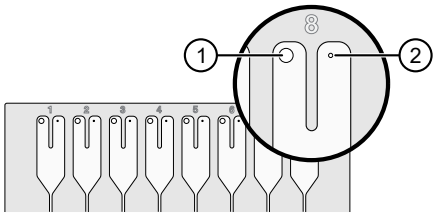


① Fill reservoirs (8 total)
② Fill reservoir strip
③ Array card carrier
④ Array card barcode
⑤ Reaction well (384 total)



① Foil
② Array card feet

The fill reservoir includes a fill port and a vent port. Use the fill port to load PCR reaction mix into the card.



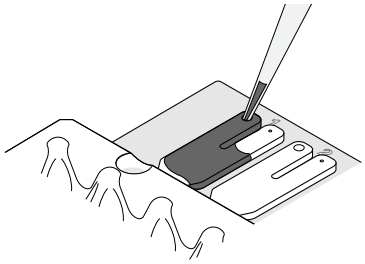
① Fill port
② Vent port

Load the PCR reaction mix

Before removing the card from its packaging:

- Prepare each sample-specific PCR reaction mix.
- Allow the card to reach room temperature.

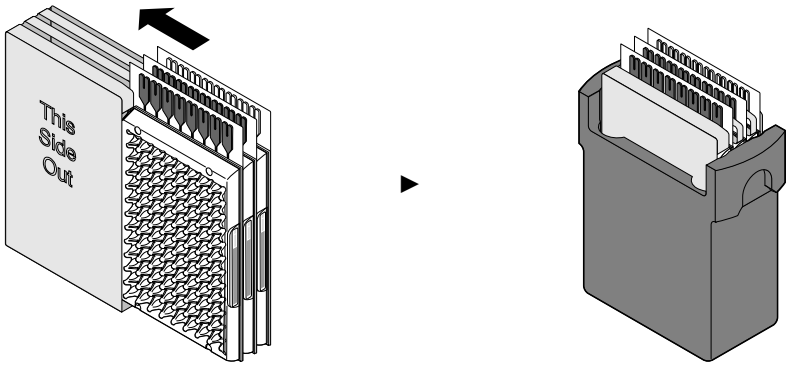
1. Carefully remove the card from its packaging.
2. Place the card on the benchtop with its foil-side down.
3. Load 100 µL of the sample-specific PCR reaction mix into a micropipette.
4. Hold the micropipette in an angled position, then place the tip into a fill port of the card.
5. Slowly dispense the entire volume of reaction mix so that it sweeps in and around the fill reservoir toward the vent port.



Centrifuge the card

1. Load the cards into the centrifuge buckets.
   a. Place the bucket on the benchtop with its label facing the front of the bench.
   b. Insert the cards into the card holder, ensuring that:
      - The fill reservoirs extend upwards out of the card holder.
      - The reaction wells face the label-side of the card holder.
   c. Insert blank balance cards into any empty positions of the card holder. All three positions in the card holder must be filled.

d. Place the loaded card holder into the bucket so that the card holder label faces the front of the bucket.



2. Configure the centrifuge using its front-panel controls.

   a. Set the bucket type to **15679**.

   b. Set the following parameters according to the control panel type.

Parameter	EASYSet (touchpad-operated)	QUIKSet (knob-operated)
Increasing ramp rate	9	3
Decreasing ramp rate	9	—
Rotational speed	1,200 rpm (331 × *g*)	1,200 rpm
Centrifugation time [1]	1 minute	1 minute

[1] You will centrifuge the cards twice, each time for 1 minute (see step 4).

IMPORTANT! A speed that is set too high can deform the card.

3. Load the buckets into the centrifuge.

   a. Press ⏏ on the centrifuge control panel to open the centrifuge cover.

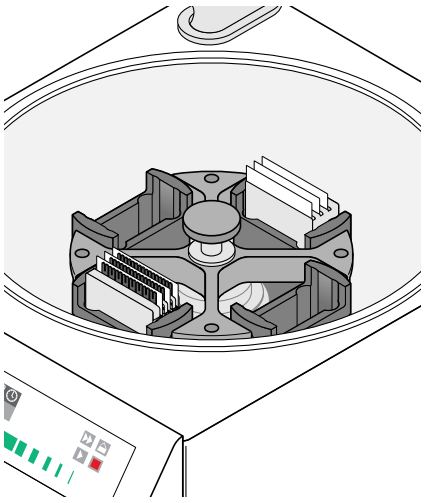
   b. Place each loaded bucket onto an open rotor arm of the centrifuge.

   Ensure that each bucket can swing easily within its slotted position on the rotor arm.

   c. If there are empty rotor arms, prepare buckets with blank balance cards as described in step 1. Place the balance buckets onto the rotor arms.

   The rotor must be evenly loaded and opposing buckets must hold the same weight.

   d. Close the centrifuge cover.



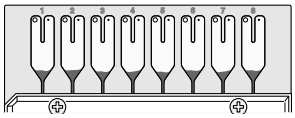
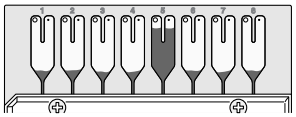
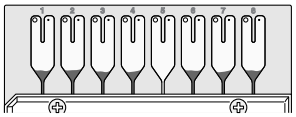
Centrifuge is properly loaded and balanced.

4. Run the centrifuge.
   a. Press ▶.
      The centrifuge will start, then automatically stop after 1 minute.
   b. Repeat substep 4a so that the cards are centrifuged for a total of two, consecutive, 1-minute centrifugations.

      IMPORTANT! Do not centrifuge the cards continuously for 2 minutes. The ramping up in speed during the *two, consecutive 1-minute* centrifugations is important for proper filling.

5. Remove the cards from the centrifuge.
   a. Press ⏏.
   b. Remove the buckets from the centrifuge, then remove the card holders from the buckets.
   c. Remove each card from the card holder by lifting it gently by the card carrier sides.

6. Examine the cards for proper filling.
   When properly filled, the remaining volumes of PCR reaction mix are consistent reservoir to reservoir.

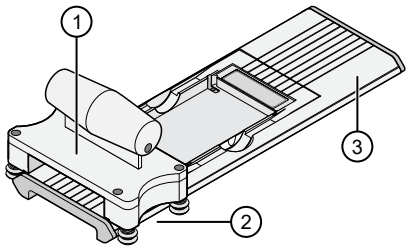
Consistent filling	Inconsistent filling	
		

Seal the card

The TaqMan™ Array Card Sealer isolates the wells of an array card after it is loaded with PCR reaction mix and centrifuged. The sealer uses a precision stylus assembly (under the carriage) to seal the main fluid distribution channels of the array card.

Note: In some documents, the TaqMan™ Array Card Sealer is referred to as a Stylus Staker.

1. Position the TaqMan™ Array Card Sealer and its carriage before inserting a card.
   a. Place the sealer on a benchtop that is approximately waist-high so that the carriage can be easily maneuvered.
   b. Position the sealer with the carriage starting position toward the front of the bench.
      Ensure that the engraved arrows on the sealer point away from you.



① Carriage
② Carriage starting position
③ Carriage ending position

c. Ensure that the carriage for the sealer is at the starting position.

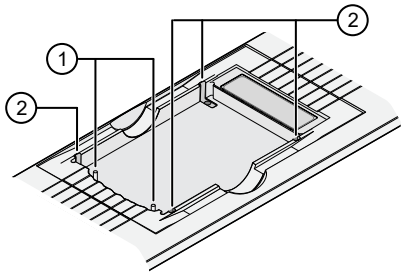
IMPORTANT! Do not insert a filled card into the sealer if the carriage is *not* in its starting position. The card will be irreparably damaged if the carriage is moved backwards across the card towards the starting position.

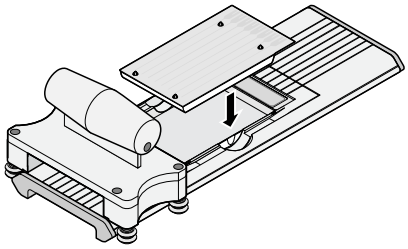
2. Insert a filled, centrifuged card into the sealer.

a. Hold the card with its foil-side up.

b. Orient the card over the sealer with the fill reservoirs of the card toward the ending position.

c. Align the rear pin grooves of the card to the alignment pins of the sealer.



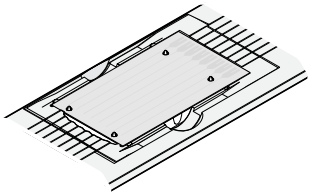


① Alignment pins
② Spring clips

d. Gently place the card on top of the sealer.

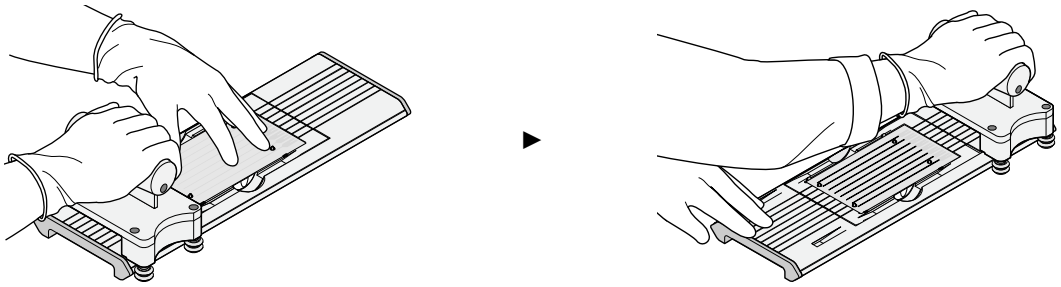
e. Gently push the card until it is seated securely in the sealer.

When properly seated, the foil surface of the card is level with the base of the sealer and the spring clips hold the card securely in place.



3. Slowly and steadily push the carriage across the sealer in the direction of the engraved arrows.

Push the carriage across the entire length of the card until the carriage meets the mechanical stops at the ending position.



IMPORTANT!

- Do not use excessive force or speed when pushing the carriage across the card.
- Do not move the carriage back across the card. Leave the carriage at the ending position while removing the card from the sealer.

4. Remove the sealed card from the sealer by grasping the sides of the card and lifting it off.

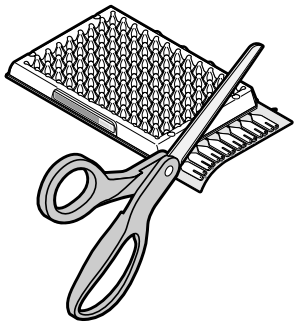
   Use the thumb slot in the middle of the sealer to access the card.

5. Examine the card for proper sealing.

   Note: When properly sealed, the indentations from the sealer carriage align with the main channels of the card.

   If the indentations do not align or if the foil is damaged, do not use the card.

6. Use scissors to trim the fill reservoir strip from the card. Use the edge of the card carrier as a guide.



IMPORTANT! Completely remove the fill reservoir strip. Any remaining plastic that extends beyond the card edge can prevent the card from seating properly on the sample block and can affect amplification.

Correct trim	Incorrect trim
	

The card is now ready to run on the instrument.

(*Optional*) Store the sealed card for up to 72 hours, protected from light, at 2°C to 8°C.

C Supplemental information

- Endogenous and exogenous controls 34
- Overview of cDNA template preparation 36
- Overview of TaqMan™ Advanced miRNA Assays chemistry 37
- Setup files for compatible instruments 39
- How to import setup files 39
- Best practices for PCR and RT-PCR experiments 40

Endogenous and exogenous controls

Endogenous controls

An endogenous control shows gene expression that is relatively constant and moderately abundant across treatment protocols, and tissues or cell types. Normalization to endogenous control genes is currently the most accurate method to correct for potential biases that are caused by:

- Sample collection
- Variation in the amount of starting material
- Reverse transcription (RT) efficiency
- Nucleic acid (RNA/DNA) preparation and quality

No single control can act as a universal endogenous control for all experimental conditions, so we recommend verifying the chosen endogenous control or set of controls for the sample tissue, cell, or treatment.

See “Endogenous and exogenous controls” on page 12 and *A technical guide to identifying miRNA normalizers using TaqMan™ Advanced miRNA Assays White Paper* (Pub. No. COL31302 0916) for available assays that target miRNAs with relatively constant expression levels across many different sample types.

Exogenous controls

An exogenous control is a synthetic RNA oligonucleotide with an miRNA target sequence that is not present in the sample of interest. For example, the target sequence for the miRNA assay ath-miR-159a is not present in humans, so it is a good exogenous control for human samples.

The RNA oligonucleotide is combined with the biological sample during the RNA isolation procedure as a spike-in control to monitor:

- Sample input amount for difficult samples (for example, serum/plasma or other biofluids).
- Extraction efficiency.

When using exogenous controls with TaqMan™ Advanced miRNA Assays:

- The assay chemistry requires that exogenous controls be 5'-phosphorylated.
- The final concentration of the spike-in control in the sample should be 1–10 pM.

See “Endogenous and exogenous controls” on page 12 for available TaqMan™ Advanced miRNA Assays which target sequences that can be used as exogenous controls with human samples.

Overview of cDNA template preparation

Quantification using TaqMan™ Advanced miRNA Assays requires the modification of mature miRNAs by the addition of a poly(A) tail (3') and an adaptor (5') to:

- Amplify all miRNAs in a single reverse transcription (RT) reaction.
- Amplify the sample for downstream PCR in a single universal cDNA reaction.

Figure 1 Poly(A) tailing reaction

Starting with a total RNA sample, poly(A) polymerase is used to add a 3'-adenosine tail to the miRNA.

Figure 2 Adaptor ligation reaction

The miRNA with poly(A) tail undergoes adaptor ligation at the 5' end. The adaptor acts as the forward-primer binding site for the miR-Amp reaction.

Figure 3 Reverse transcription (RT) reaction

A Universal RT primer binds to the 3' poly(A) tail and the miRNA is reverse transcribed. The resulting cDNA is suitable for all TaqMan™ Advanced miRNA Assays.

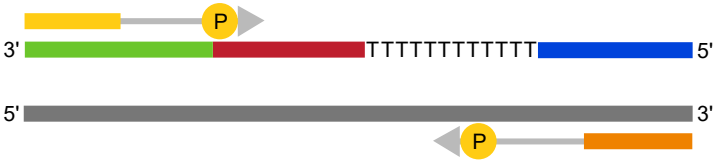


Figure 4 miR-Amp reaction

Universal forward and reverse primers increase the number of cDNA molecules.

Overview of TaqMan™ Advanced miRNA Assays chemistry

TaqMan™ MGB probes

TaqMan™ MGB probes contain:

- A reporter dye (for example, FAM™ dye) at the 5′ end of the probe.
- A non-fluorescent quencher (NFQ) dye at the 3′ end of the probe.

  The NFQ dye does not fluoresce, which allows the real-time PCR system to measure the reporter dye contributions more accurately.
- A minor groove binder (MGB) at the 3′ end of the probe that:
  - Increases the melting temperature (T_m) without increasing the probe length.
  - Allows for the design of shorter probes.

About the 5' nuclease assay

Note: The following figures are general representations of real-time PCR with TaqMan™ MGB probes and TaqMan™ Advanced miRNA Assays. The sequence regions are not necessarily drawn to scale.

The 5' nuclease assay process takes place during PCR amplification. It occurs in every cycle and does not interfere with the exponential accumulation of product.

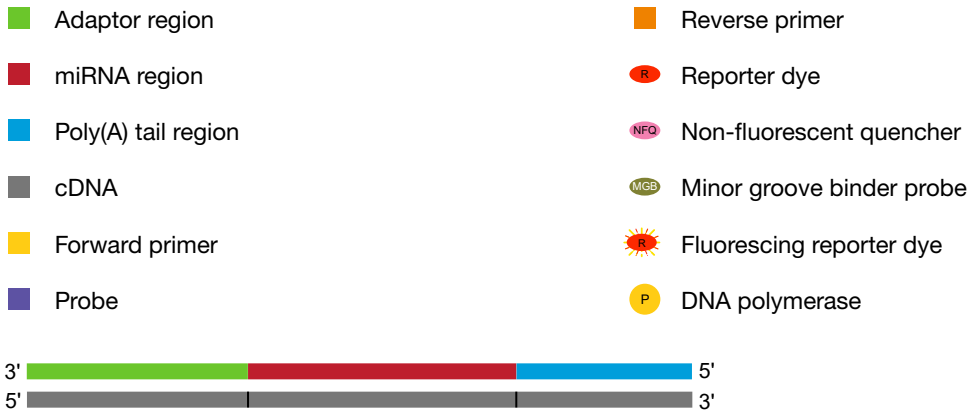


Figure 5 cDNA synthesis product

During the PCR, the forward and reverse primers anneal to complementary sequences along the denatured cDNA template strands (Figure 6). The primer binding sites vary depending on the target miRNA sequence and are designed to maximize specificity. Figure 6 shows an example representation in which the reverse primer is the primer that partially overlaps the miRNA region.

The TaqMan™ MGB probe anneals specifically to a complementary sequence between the forward and reverse primer sites (Figure 6). When the probe is intact, the proximity of the reporter dye and quencher dye suppresses the reporter fluorescence, primarily by Förster-type energy transfer.

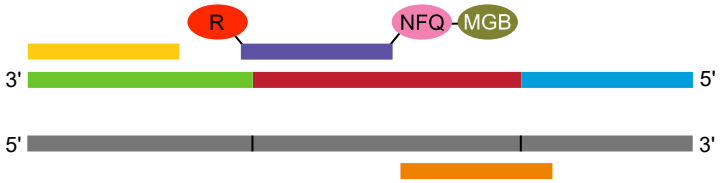


Figure 6 Annealing of probes and primers to cDNA strands

During polymerization, the DNA polymerase cleaves only probes that hybridize to the target sequence. Cleavage separates the reporter dye from the probe. The separation of the reporter dye from the quencher dye results in increased fluorescence by the reporter dye (Figure 7).

This increase in fluorescence occurs only if the probe is complementary to the target sequence and if the target sequence is amplified during PCR. Because of these conditions, nonspecific amplification is not detected.

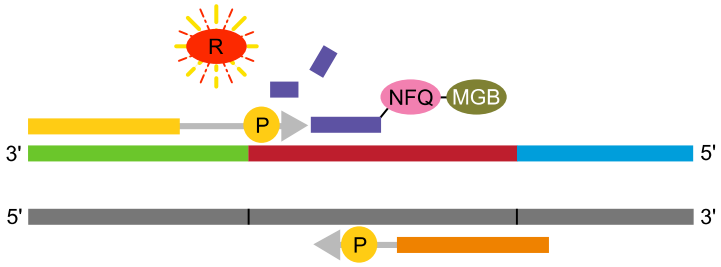


Figure 7 Initial polymerization and cleavage of reporter dye

Polymerization of the strand continues (Figure 8), but because the 3' end of the probe is blocked, no extension of the probe occurs during PCR.

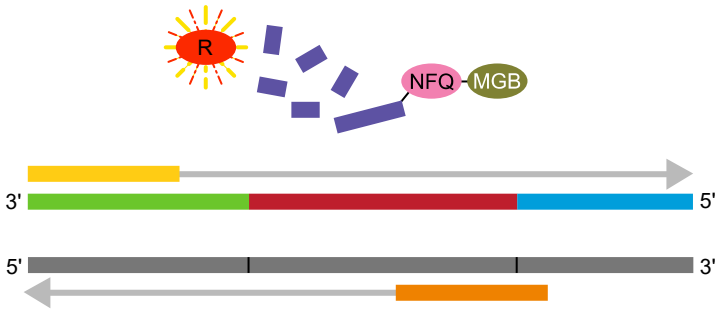


Figure 8 Completion of polymerization

Setup files for compatible instruments

Instrument	Setup file
QuantStudio™ 7 Flex System	`<ordernumber>_SDS_QS_ADV MIRNA card.txt` `<ordernumber>_SDS_QS MIRNA card.txt`
QuantStudio™ 7 Pro Real-Time PCR System	
QuantStudio™ 12K Flex Real–Time PCR System	
ViiA™ 7 Real-Time PCR System	`<ordernumber>_SDS_VIIA7_ADV MIRNA card.txt` `<ordernumber>_SDS_VIIA7 MIRNA card.txt`
7900HT Fast Real-Time PCR System	`<ordernumber>_SDS_ADV MIRNA card.txt`

How to import setup files

Instrument	Navigation
QuantStudio™ 7 Pro Real-Time PCR System	1. In QuantStudio™ Design and Analysis Software v2, navigate to the **Plate Setup** tab and the plate layout pane. 2. Click ••• **(Actions)** ▸ **Import Plate Setup**. 3. Navigate to, then select the file. 4. Click **Open**.
QuantStudio™ 7 Flex System	In the menu bar during setup, select **File** ▸ **Import Plate Setup**.
QuantStudio™ 12K Flex Real–Time PCR System	
ViiA™ 7 Real-Time PCR System	
7900HT Fast Real-Time PCR System	In the menu bar, select **File** ▸ **New Plate Wizard**, then follow the prompts.

Best practices for PCR and RT-PCR experiments

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

Use UNG to prevent false-positive amplification

Carryover amplicons can result in false-positive amplification during PCR. Use a master mix that contains heat-labile uracil-N-glycosylase (UNG; also known as uracil-DNA glycosylase (UDG)) to degrade many contaminating carryover amplicons.

UNG enzymatic activity occurs during the PCR reaction setup at room temperature; an activation step before thermal cycling is not necessary. Unlike standard UNG, heat-labile UNG is completely inactivated during the first ramp to the high-temperature step for template denaturation and polymerase activation.

To ensure the desired UNG activity:

- Use PCR components and thermal cycling conditions as specified.

  UNG-containing master mixes incorporate the optimal concentration of UNG to prevent cross-contamination while not affecting real-time PCR performance.
- Do not attempt to use UNG-containing master mixes in subsequent amplification of dU-containing PCR products, such as in nested-PCR protocols. The UNG will degrade the dU-containing PCR products, preventing further amplification.

Although treatment with UNG can degrade or eliminate large numbers of carryover PCR products, use good laboratory practices to minimize cross-contamination from non-dU-containing PCR products or other samples.

Detect fluorescent contaminants

Fluorescent contaminants can generate false positive results. To help detect these contaminants, we recommend including a no-amplification control reaction that contains sample, but no master mix.

After PCR, if the absolute fluorescence of the no-amplification control is greater than the fluorescence of the no template control (NTC), fluorescent contaminants may be present in the sample or in the heat block of the real-time PCR instrument.

D

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

E Documentation and support

Related documentation

Document	Pub. No.
TaqMan™ Advanced miRNA Assays Quick Reference—TaqMan™ Array Cards	MAN0016123
TaqMan™ Advanced miRNA cDNA Synthesis Kit Product Information Sheet	MAN0011141
Understanding Your Shipment For detailed information about the Assay Information File (AIF)	MAN0017153
A technical guide to identifying miRNA normalizers using TaqMan™ Advanced miRNA Assays	COL31302 0916[1]
QuantStudio™ Design and Analysis Software v2 User Guide	MAN0018200
QuantStudio™ 7 Pro Real-Time PCR System	
QuantStudio™ 6 Pro Real-Time PCR System and QuantStudio™ 7 Pro Real-Time PCR System User Guide	MAN0018045
QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide	4489821
QuantStudio™ Real-Time PCR Software Getting Started Guide	4489822
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.6.1 or later) Maintenance and Administration Guide	MAN0018828
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.6.1 or later) Quick Reference	MAN0018829
QuantStudio™ 12K Flex Real–Time PCR System	
QuantStudio™ 12K Flex Real–Time PCR System Maintenance and Administration Guide	4470689
QuantStudio™ 12K Flex Real–Time PCR System: Multi-Well Plates and Array Card Experiments User Guide	4470050
ViiA™ 7 Real-Time PCR System	
Applied Biosystems™ ViiA™ 7 Real-Time PCR System User Guide: Calibration, Maintenance, Networking, and Security	4442661
Applied Biosystems™ ViiA™ 7 Real-Time PCR System Getting Started Guide	4441434

(continued)

Document	Pub. No.
7900HT Real-Time PCR System	
Applied Biosystems™ 7900HT Fast Real-Time PCR System Maintenance and Troubleshooting Guide	4365542
Applied Biosystems™ 7900HT Fast Real-Time PCR System User Bulletin	4352533

[1] Available at **thermofisher.com/advancedmirna** (in the **Resources** section).

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.