TaqMan™ Gene Expression Assays—TaqMan™ Array Plates

USER GUIDE

96-well Standard (0.2-mL) TaqMan™ Array Plates
96-well Fast (0.1-mL) TaqMan™ Array Plates

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Product description

The procedures in this document are for use with TaqMan™ Gene Expression Assays configured and preplated on TaqMan™ Array Plates.

TaqMan™ Gene Expression Assays are a comprehensive collection of predesigned, preformulated primer and probe sets to perform quantitative gene expression studies on a variety of species.

For a current list of available species and assays, use the assay search tool at thermofisher.com/taqmangeneexpression.

- TaqMan™ Gene Expression Assays
  - A general collection of assays that target protein-coding transcripts from a variety of species and for specific diseases, pathways, or biological processes.
  - TaqMan™ Non-coding RNA Assays that target long non-coding RNA (ncRNA) in human, mouse, and rat species. These assays are designed for ncRNAs that are > 60 nt in length.

- Endogenous control assays (see page 23 for more information)

The following configurations are available.

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Description</th>
<th>Customizable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed-content TaqMan™ Array Plates</td>
<td>Preplated and predefined TaqMan™ Gene Expression Assays that are manufactured and stocked in advance</td>
<td>No</td>
</tr>
<tr>
<td>Flexible-content TaqMan™ Array Plates</td>
<td>Plates configured with a suggested selection of TaqMan™ Gene Expression Assays, categorized by disease, pathway, or biological process</td>
<td>Preselected assays can be substituted with other predesigned assays that target TaqMan™ Gene Expression Assays more applicable to experiment needs</td>
</tr>
</tbody>
</table>
### Configuration Table

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Description</th>
<th>Customizable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Custom TaqMan™ Array Plates</td>
<td>Fully customizable plates</td>
<td>Allows the configuration of the TaqMan™ Array Plates with any predesigned assays</td>
</tr>
<tr>
<td>TaqMan™ Array Plates with RFID</td>
<td>Fully customizable plates</td>
<td></td>
</tr>
</tbody>
</table>

This document provides guidance for preparing cDNA templates (see page 12) and protocols for performing real-time PCR using a variety of compatible instruments and Master Mixes (see page 13 and page 27).

For detailed information about TaqMan™ Gene Expression Assays, see page 23.

### About TaqMan™ Array Plates with RFID

TaqMan™ Array Plates with RFID are specialized plates that are configured with an RFID tag that the QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems can identify. The tag contains the necessary information to start a run immediately after the plate is loaded into the instrument.

After the tagged plate is loaded, the instrument reads the RFID tag and uploads the listed information.

- Plate type
- Plate barcode, expiration date, catalog number, and lot number
- Reaction volume per well
- Assay targets
- Passive reference dye (ROX™ dye)
- Thermal protocol (fast mode)
Contents and storage

TaqMan™ Array Plates and TaqMan™ Array Plates with RFID contain dried-down TaqMan™ Gene Expression Assays according to the configured plate layout.

TaqMan™ Array Plates with RFID are ordered by adding an RFID (Cat. No. A43823) to any of the standard plate catalog numbers listed in Table 1 on page 7. For information and run instructions see, Appendix B, “Detailed procedures for TaqMan™ Array Plates with RFID”.

Table 1  Available plate layouts for flexible-content and Custom TaqMan™ Array Plates

<table>
<thead>
<tr>
<th>Format</th>
<th>Cat. No. (Standard, 0.2-mL)</th>
<th>Cat. No. (Fast, 0.1-mL)</th>
<th>Number of assays + controls</th>
<th>Number of samples</th>
<th>Storage¹</th>
<th>¹See packaging for expiration date. ²Shipped at ambient temperature. See thermofisher.com/ambientshipping. ³TaqMan™ “Plus” plates only use human endogenous controls; controls for other species are not supported.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TaqMan™ Array Plate 8</td>
<td>4413266</td>
<td>4413263</td>
<td>7+ 1</td>
<td>12</td>
<td></td>
<td>15–30°C²</td>
</tr>
<tr>
<td>TaqMan™ Array Plate 16</td>
<td>4413264</td>
<td>4413261</td>
<td>15+ 1</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TaqMan™ Array Plate 16  Plus³</td>
<td>4413265</td>
<td>4413262</td>
<td>12+ 4</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TaqMan™ Array Plate 32</td>
<td>4391528</td>
<td>4413259</td>
<td>31+ 1</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TaqMan™ Array Plate 32  Plus³</td>
<td>4391529</td>
<td>4413260</td>
<td>28+ 4</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TaqMan™ Array Plate 48</td>
<td>4391526</td>
<td>4413257</td>
<td>47+ 1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TaqMan™ Array Plate 48  Plus³</td>
<td>4391527</td>
<td>4413258</td>
<td>44+ 4</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TaqMan™ Array Plate 96</td>
<td>4391524</td>
<td>4413255</td>
<td>95+ 1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TaqMan™ Array Plate 96  Plus³</td>
<td>4391525</td>
<td>4413256</td>
<td>92+ 4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 plate. The HTML and CSV files contain identical information.
- **Setup files (SDS in TXT format)**—Contain the sample setup on the plate. The files are imported into the instrument software to perform real-time PCR.
- **Assay Information File (AIF in TXT format)**—Describes the TaqMan™ Gene Expression Assays. See Understanding Your Shipment (Pub. No. MAN0017153) for more 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.
Order TaqMan™ Array Plates

- Order fixed-content TaqMan™ Array Plates.
  a. Go to thermofisher.com/taqmanarrays.
  b. Select preconfigured content by disease, pathway, biological process, or gene symbol.

- Order flexible-content TaqMan™ Array Plates.
  a. Go to thermofisher.com/flexiblepanels.
  b. Browse by panel, then by species.

- Order custom-configured TaqMan™ Array Plates.
  a. Go to thermofisher.com/arrayplates.
  b. Follow the prompts to configure the plate.
  c. (Optional) For TaqMan™ Array Plates with RFID, request an RFID (Cat. No. A43823) to be added to your order.
### Required materials and equipment not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](http://thermofisher.com). MLS: Fisher Scientific ([fisherscientific.com](http://fisherscientific.com)) or other major laboratory supplier.

#### Table 2  Recommended products for isolation of RNA

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kits for RNA isolation</td>
<td>thermofisher.com/rnaisolation</td>
</tr>
</tbody>
</table>

#### Table 3  Recommended products for preparation of cDNA

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>cDNA kit or cDNA Master Mix, one of the following:</td>
<td></td>
</tr>
<tr>
<td>SuperScript™ IV Vilo™ Master Mix</td>
<td>11756050</td>
</tr>
<tr>
<td>SuperScript™ IV Vilo™ Master Mix with ezDNAse™ Enzyme</td>
<td>11766050</td>
</tr>
<tr>
<td>High-Capacity cDNA Reverse Transcription Kit</td>
<td>4368813</td>
</tr>
</tbody>
</table>

#### Table 4  PCR Master Mixes

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Recommended) TaqMan™ Fast Advanced Master Mix</td>
<td>4444556</td>
</tr>
<tr>
<td>TaqMan™ Gene Expression Master Mix</td>
<td>4369016</td>
</tr>
<tr>
<td>TaqMan™ Universal Master Mix II, with UNG</td>
<td>4440038</td>
</tr>
<tr>
<td>TaqMan™ Universal Master Mix II, no UNG</td>
<td>4440047</td>
</tr>
<tr>
<td>TaqMan™ Fast Universal PCR Master Mix, no AmpErase™ UNG</td>
<td>4352042</td>
</tr>
</tbody>
</table>

#### Table 5  Other materials and equipment required for the workflow

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real-time PCR instrument, one of the following:</td>
<td>Contact your local sales office</td>
</tr>
<tr>
<td>QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems</td>
<td></td>
</tr>
<tr>
<td>QuantStudio™ 3 or 5 Real-Time PCR System</td>
<td></td>
</tr>
<tr>
<td>QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System</td>
<td></td>
</tr>
<tr>
<td>QuantStudio™ 12K Flex Real-Time PCR System</td>
<td></td>
</tr>
<tr>
<td>StepOnePlus™ Real-Time PCR System</td>
<td></td>
</tr>
<tr>
<td>Viia™ 7 Real-Time PCR System</td>
<td></td>
</tr>
<tr>
<td>7500/7500 Fast Real-Time PCR System</td>
<td></td>
</tr>
<tr>
<td>7900HT Fast Real-Time PCR System</td>
<td></td>
</tr>
</tbody>
</table>
### Chapter 1 Product information

**Required materials and equipment not supplied**

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Software</strong></td>
<td></td>
</tr>
<tr>
<td><em>(Optional)</em> Relative Quantification application</td>
<td>Available on the Connect platform</td>
</tr>
<tr>
<td><em>(Optional)</em> ExpressionSuite™ Software</td>
<td>Available at thermofisher.com/expressionsuite</td>
</tr>
<tr>
<td><strong>Equipment</strong></td>
<td></td>
</tr>
<tr>
<td>Thermal cycler, one of the following (or equivalent):</td>
<td></td>
</tr>
<tr>
<td>• Thermal Cycler</td>
<td>Contact your local sales office</td>
</tr>
<tr>
<td>• SimpliAmp™ Thermal Cycler</td>
<td></td>
</tr>
<tr>
<td>• ProFlex™ PCR System</td>
<td></td>
</tr>
<tr>
<td>Centrifuge, with adapter for 96-well plates</td>
<td>MLS</td>
</tr>
<tr>
<td>Microcentrifuge</td>
<td>MLS</td>
</tr>
<tr>
<td>Vortex mixer</td>
<td>MLS</td>
</tr>
<tr>
<td><em>(Optional)</em> Eppendorf™ MixMate™ (shaker)</td>
<td>Fisher Scientific™ 21-379-00</td>
</tr>
<tr>
<td>Pipettes</td>
<td>MLS</td>
</tr>
<tr>
<td><strong>Tubes, plates, and other consumables</strong></td>
<td>thermofisher.com/plastics</td>
</tr>
<tr>
<td>Tubes, plates, and film</td>
<td></td>
</tr>
<tr>
<td>Aerosol-resistant barrier pipette tips</td>
<td>MLS</td>
</tr>
<tr>
<td>Disposable gloves</td>
<td>MLS</td>
</tr>
<tr>
<td><strong>Reagents</strong></td>
<td></td>
</tr>
<tr>
<td>Nuclease-free Water</td>
<td>AM9930</td>
</tr>
<tr>
<td>RNase Inhibitor</td>
<td>N8080119</td>
</tr>
<tr>
<td>RNaseOUT™ Recombinant Ribonuclease Inhibitor</td>
<td>10777019</td>
</tr>
<tr>
<td>TURBO DNA-free™ Kit</td>
<td>AM1907</td>
</tr>
<tr>
<td><em>(Optional)</em> TaqMan™ PreAmp Master Mix</td>
<td>4391128</td>
</tr>
<tr>
<td><em>(Optional)</em> TaqMan™ PreAmp Master Mix Kit</td>
<td>4384267</td>
</tr>
</tbody>
</table>
Workflow

Start with cDNA templates from RNA samples (page 12)

▼

Combine cDNA and Master Mix [page 14]

▼

Prepare the TaqMan™ Array Plate (page 14)

▼

Set up and run the real-time PCR instrument (page 15)

▼

Analyze the results (page 17)
Guidelines for preparation of cDNA

Guidelines for isolation of high-quality RNA

- See Table 2 on page 9 for recommended RNA isolation kits.
- (Optional) Use DNase to ensure minimal genomic DNA contamination of the RNA.

Guidelines for preparing cDNA templates

- See Table 3 on page 9 for recommended cDNA synthesis kits.
- Use the same reverse transcription procedure for all samples.
- 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

  **Note:** We recommend using RNase Inhibitor (Cat. No. N8080119) or RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777019).
  - Nondegraded total RNA (not applicable for double-stranded templates)

  **IMPORTANT!** Degradation of the RNA may reduce the yield of cDNA for some gene targets.

- For the input RNA amount, follow the recommendations provided by the cDNA kit.
- Small amounts of cDNA can be pre-amplified. Use TaqMan™ PreAmp Master Mix (Cat. No. 4391128) or TaqMan™ PreAmp Master Mix Kit (Cat. No. 4384267).
- Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes, then include 10% overage, unless otherwise indicated.
- If using strip tubes to prepare cDNA templates, change to a new cap after each step or incubation.
- See your instrument user guide for detailed instructions about using plates, tubes, or strip tubes to prepare cDNA templates.
Perform real-time PCR

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- Analyze the results .................................................. 17

Procedural guidelines for performing real-time PCR

- Follow best-practices when preparing or performing PCR (see page 29).
- 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 user documents.
- Keep the plate 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 plate.
Combine cDNA and Master Mix

Thaw the cDNA samples on ice. Resuspend the cDNA samples by inverting the tube, then gently vortexing.

1. Mix the Master Mix thoroughly but gently.

2. Combine the cDNA and Master Mix in an appropriately-sized microcentrifuge tube.
   Use one of the following tables.

<table>
<thead>
<tr>
<th>Table 6</th>
<th>96-well Standard (0.2-mL) Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component</td>
<td>Volume</td>
</tr>
<tr>
<td>cDNA sample + nuclease-free water[2]</td>
<td>10 µL</td>
</tr>
<tr>
<td>Master Mix (2X)[3]</td>
<td>10 µL</td>
</tr>
<tr>
<td>Total volume</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

[1] Includes 12.5% overage.
[2] Ensure that the final cDNA concentration per well is 1–100 ng per 20-µL reaction.

<table>
<thead>
<tr>
<th>Table 7</th>
<th>96-well Fast (0.1-mL) Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component</td>
<td>Volume</td>
</tr>
<tr>
<td>cDNA sample + nuclease-free water[2]</td>
<td>5 µL</td>
</tr>
<tr>
<td>Master Mix (2X)[3]</td>
<td>5 µL</td>
</tr>
<tr>
<td>Total volume</td>
<td>10 µL</td>
</tr>
</tbody>
</table>

[1] Includes 12.5% overage.
[2] Ensure that the final cDNA concentration per well is 5–50 ng per 10-µL reaction.

3. Vortex the tube to mix the contents thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.

Prepare the TaqMan™ Array Plate

1. Remove the plate from its packaging, centrifuge briefly, then remove the plate cover.

2. Add the cDNA-Master Mix to the appropriate wells of the plate.
   - 96-well Standard (0.2-mL) Plate: 20 µL per well
   - 96-well Fast (0.1-mL) Plate: 10 µL per well
IMPORTANT! For optimal results when using TaqMan™ Fast Universal PCR Master Mix, no AmpErase™ UNG, prepare the plate on ice. Run the plate within 2 hours of preparation, or store the plate at 2–8°C for up to 24 hours.

3. Seal the plate with MicroAmp™ Optical Adhesive Film, then vortex briefly to mix the contents.

4. Centrifug e the plate briefly to collect the contents to the bottom of the wells.

Set up and run the real-time PCR instrument

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

For instructions on how to set up and run a TaqMan™ Array Plate with RFID on the QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems see, Appendix B, “Detailed procedures for TaqMan™ Array Plates with RFID”.

Note: The instrument must be configured with the block appropriate for the plate type.

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

   See “Import the setup files” on page 26 for instructions to import setup files.

   Note: The setup files are instrument-specific. See “Instrument software and setup files” on page 26 for more information.

2. Select the cycling mode appropriate for the Master Mix.

   IMPORTANT! The cycling mode depends on the Master Mix that is used in the reaction. The cycling mode does not depend on a Standard or a Fast plate format.
3. Set up the thermal protocol for your instrument.

   See “Thermal protocols” on page 27 for the thermal protocols for other Master Mixes.

   **Table 8** TaqMan™ Fast Advanced Master Mix (StepOnePlus™, ViiA™ 7, and QuantStudio™ systems with fast cycling mode)

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNG incubation[1]</td>
<td>50°C</td>
<td>2 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Enzyme activation</td>
<td>95°C</td>
<td>20 seconds[2]</td>
<td>1</td>
</tr>
<tr>
<td>Denature</td>
<td>95°C</td>
<td>1 second</td>
<td>40</td>
</tr>
<tr>
<td>Anneal / Extend</td>
<td>60°C</td>
<td>20 seconds</td>
<td></td>
</tr>
</tbody>
</table>

   [1] Optional, for optimal UNG activity.

   [2] Enzyme activation can be up to 2 minutes. The time should not cause different results. See Enzyme activation time.

   **Table 9** TaqMan™ Fast Advanced Master Mix (7500 and 7500 Fast systems with fast cycling mode)

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNG incubation[1]</td>
<td>50°C</td>
<td>2 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Enzyme activation</td>
<td>95°C</td>
<td>20 seconds[2]</td>
<td>1</td>
</tr>
<tr>
<td>Denature</td>
<td>95°C</td>
<td>3 seconds</td>
<td>40</td>
</tr>
<tr>
<td>Anneal / Extend</td>
<td>60°C</td>
<td>30 seconds</td>
<td></td>
</tr>
</tbody>
</table>

   [1] Optional, for optimal UNG activity.

   [2] Enzyme activation can be up to 2 minutes. The time should not cause different results. See Enzyme activation time.

4. Set the reaction volume appropriate for the reaction plate.

   • 96-well Standard (0.2-mL) Plate: 20 µL
   • 96-well Fast (0.1-mL) Plate: 10 µL

5. Load the plate into the real-time PCR instrument.

6. Start the run.
Analyze the results

For detailed information about data analysis, see the appropriate documentation for your instrument.

Use the relative quantification (ΔΔC\text{t}) method to analyze results.

The general guidelines for analysis include:

- View the amplification plot; then, if needed:
  - Adjust the baseline and threshold values.
  - Remove outliers from the analysis.
- In the well table or results table, view the C\text{t} values for each well and for each replicate group.

Perform additional data analysis using any of the following software:

<table>
<thead>
<tr>
<th>Software</th>
<th>Resource</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Quantification application</td>
<td>thermofisher.com/connect</td>
</tr>
<tr>
<td>ExpressionSuite™ Software\textsuperscript{[1]}</td>
<td>thermofisher.com/expressionsuite</td>
</tr>
</tbody>
</table>

\textsuperscript{[1]} Can automatically define the baseline.

For more information about real-time PCR, see “Procedural guidelines for performing real-time PCR” on page 13) or go to thermofisher.com/qpcReducation.

Data can be analyzed using the relative threshold algorithm (C\text{rt}).

See “Algorithms for data analysis“ on page 27.

Use the relative threshold algorithm in your software. If your software does not have the relative threshold algorithm, you can use the Relative Quantification application that is available on Connect (thermofisher.com/connect).

See Introduction to Gene Expression Getting Started Guide (Pub. No. 4454239) for information about C\text{t} and C\text{rt}. 
Troubleshooting and FAQs

Visit our online FAQ database for tips and tricks for conducting your experiment, troubleshooting information, and FAQs. The online FAQ database is frequently updated to ensure accurate and thorough content.

• For troubleshooting information and FAQs for this product: thermofisher.com/taqmanarrayplatesfaqs
• To browse the database and search using keywords: thermofisher.com/faqs
Detailed procedures for TaqMan™ Array Plates with RFID

- Load the plate ....................................................... 19
- (Optional) Edit thermal protocol ................................. 20
- Run the plate ........................................................ 20
- Unload a plate from the instrument .............................. 21
- Optional methods for TaqMan™ Array Plates with RFID .......... 21

This section provides instructions for the use of a TaqMan™ Array Plate with RFID on the QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems. For detailed instrument instructions, supplemental procedures, and troubleshooting, see the QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems User Guide (Pub. No. MAN0018045).

Load the plate

IMPORTANT! The instrument should be used by trained operators who have been warned of the moving parts hazard.

1. Tap (Eject) to open the instrument drawer.

2. Load the plate onto the block.
   - Well A1 of the plate is in the top-left corner of the block.
   - The barcode faces the front of the instrument.
   - The RFID tag is on the A1 side of the plate.

3. Tap (Eject) to close the instrument drawer.

The instrument displays the Plate Properties screen.
(Optional) Edit thermal protocol

The RFID tag is configured with a fast thermal protocol and can be edited on the instrument before a run.

**IMPORTANT!** The cycling mode depends on the Master Mix that is used in the reaction. A Fast Master Mix is compatible with standard thermal protocol, but a Standard Master Mix is not compatible with a fast thermal protocol. For examples of thermal protocols see, “Thermal protocols” on page 27.

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNG Incubation</td>
<td>50°C</td>
<td>2 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Enzyme Activation</td>
<td>95°C</td>
<td>20 seconds</td>
<td>1</td>
</tr>
<tr>
<td>Denature</td>
<td>95°C</td>
<td>1 second</td>
<td>40</td>
</tr>
<tr>
<td>Anneal/Extend</td>
<td>60°C</td>
<td>20 seconds</td>
<td></td>
</tr>
</tbody>
</table>

1. In the Method screen, tap **Edit**.
2. Tap a field, then enter the changes.
   - Cover temperature
   - Reaction volume
   - Temperature for any steps
   - Time for any steps
   - Number of PCR cycles
3. Tap **Save**, then select the storage location for the plate file.
4. Tap **Done**.

Run the plate

1. *(Optional)* Edit the passive reference dye.
   **Note:** The default passive reference is ROX™ dye.
2. *(Optional)* In the Plate properties screen, edit the Run Data File Name.
3. Tap the location to send the data file.
   - ✨ Connect
     **Note:** You must be signed in with a Connect profile.
   - 🌌 USB Device
   - 🌌 Network Drive
• • This Instrument

   Note: This option is not available if you are using the instrument as a guest. You must be signed in.

   The connection status of each location of location is displayed.

4. (Optional) Deselect C_q Export.
   A separate CSV file is generated that shows the C_q calculation for each target when this option is selected.

5. Tap Start run.

   The status dial is displayed. The status dial contains the following information:
   • The block temperature
   • The elapsed time of the run
   • The status of the run

Unload a plate from the instrument

⚠️ CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the plate temperature can reach 100°C. Allow it to cool to room temperature before handling.

   The status dial displays Run complete when the run is finished. If the automatic sign-out feature is enabled and the set time has elapsed, the instrument will display the Sign In screen.

   1. Tap (Eject) to open the instrument drawer, then remove the plate.

   2. Tap (Eject) to close the instrument drawer.

   For information on plate disposal, see “Chemical safety” on page 31 and “Biological hazard safety” on page 32.

Optional methods for TaqMan™ Array Plates with RFID

Edit wells manually

   A sample setup file can be imported instead of assigning or editing a well manually (see “Import a sample setup” on page 22).

   Use the plate view ( ) to edit a well.

   1. In the Plate tab, tap the Sample Information pane, then tap Samples.

      Note: If wells are not selected, the pane is labeled Legend.

   2. Enter a sample name.
3. Select a sample type from the dropdown list.
   - Unknown
   - Standard
   - No template control (NTC)

4. Tap Done.

Import a sample setup

This allows you to import a sample setup file. This file shows the position of each sample on the plate.

1. In the Properties tab, tap Actions, then tap Import sample setup.

2. In the Import Sample Setup screen, tap the appropriate folder, then tap the appropriate file.

Apply a different setup to the plate file

This feature allows you to use a custom plate file with the data on the RFID tag.

1. In the Plate Properties screen, in the Properties tab, tap Actions ➔ Apply Different Setup.

2. Tap the location of the plate file in the left column, then tap the template file in the right column.

3. Tap Apply.
Endogenous controls

An endogenous control shows gene expression that is relatively constant and moderately abundant across tissues and cell types and treatment protocols. 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 Using TaqMan™ Endogenous Control Assays to select an endogenous control for experimental studies (Pub. No. COL33019 0619), available from thermofisher.com.

To select and order endogenous control assays, go to thermofisher.com/taqmancontrols.
**TaqMan™ Gene Expression Assays chemistry overview**

**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™ Gene Expression 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 cDNA synthesis product.

- **cDNA template**
- **Reporter dye**
- **Target region of cDNA template**
- **Fluorescence signal by reporter dye**
- **Forward primer**
- **Quencher dye**
- **Reverse primer**
- **MGB**
- **Probe**

**Figure 1** cDNA synthesis product

During the PCR, the forward and reverse primers anneal to complementary sequences along the denatured cDNA template strands (see Figure 2).

The TaqMan™ MGB probe anneals specifically to a complementary sequence between the forward and reverse primer sites (see Figure 2). 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 2** Annealing of probes and primers to cDNA strands

During polymerization, the DNA polymerase only cleaves 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 (see Figure 3).
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 3](image1)

**Figure 3** Initial polymerization and cleavage of reporter dye

Polymerization of the strand continues (see Figure 4), but because the 3’ end of the probe is blocked, no extension of the probe occurs during PCR.

![Figure 4](image2)

**Figure 4** Completion of polymerization

### Enzyme activation time

Using TaqMan™ Fast Advanced Master Mix, the enzyme activation step can range from 20 seconds to 2 minutes. A 20-second enzyme activation step is sufficient when the template is cDNA. A longer enzyme activation time will not affect the results.

The enzyme activation time for the default fast thermal cycling conditions on the instruments is 20 seconds. If a longer enzyme activation time is required, change the thermal cycling conditions before starting the run. A longer enzyme activation time can help to denature double-stranded genomic DNA when genomic DNA is used.
## Instrument software and setup files

<table>
<thead>
<tr>
<th>Instrument software</th>
<th>Setup file</th>
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</thead>
<tbody>
<tr>
<td>QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems</td>
<td>ordernumber_7500_2.0_FAST.txt</td>
</tr>
<tr>
<td>QuantStudio™ 3 or 5 Real-Time PCR System</td>
<td></td>
</tr>
<tr>
<td>QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System</td>
<td>ordernumber_StepOne_2.1.txt</td>
</tr>
<tr>
<td>QuantStudio™ 12K Flex Real-Time PCR System</td>
<td>ordernumber_7500_2.0_FAST.txt</td>
</tr>
<tr>
<td>Viia™ 7 Real-Time PCR System</td>
<td>ordernumber_7300_7500_SDS.txt</td>
</tr>
<tr>
<td>StepOnePlus™ Real-Time PCR System</td>
<td></td>
</tr>
<tr>
<td>7500/7500 Fast Real-Time PCR System [v2.0]</td>
<td>ordernumber_7500_2.0_FAST.txt</td>
</tr>
<tr>
<td>7500/7500 Fast Real-Time PCR System [v1.0]</td>
<td>ordernumber_7300_7500_SDS.txt</td>
</tr>
</tbody>
</table>

## Import the setup files

<table>
<thead>
<tr>
<th>Real-time PCR instrument</th>
<th>Navigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems</td>
<td>In the menu bar during setup, select <strong>File &gt; Import Plate Setup.</strong></td>
</tr>
<tr>
<td>QuantStudio™ 3 or 5 Real-Time PCR System</td>
<td></td>
</tr>
<tr>
<td>QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System</td>
<td>In the menu bar, select <strong>File &gt; Import.</strong></td>
</tr>
<tr>
<td>QuantStudio™ 12K Flex Real-Time PCR System</td>
<td></td>
</tr>
<tr>
<td>Viia™ 7 Real-Time PCR System</td>
<td></td>
</tr>
<tr>
<td>StepOnePlus™ Real-Time PCR System</td>
<td>In the menu bar, select <strong>Plate Setup &gt; File &gt; Import.</strong></td>
</tr>
<tr>
<td>7500/7500 Fast Real-Time PCR System [v2.0]</td>
<td>In the menu bar, select <strong>File &gt; Import Sample Setup.</strong></td>
</tr>
<tr>
<td>7500/7500 Fast Real-Time PCR System [v1.0]</td>
<td></td>
</tr>
</tbody>
</table>
Algorithms for data analysis

Table 10  Algorithm recommendations for TaqMan™ Array Plates

<table>
<thead>
<tr>
<th>Algorithm</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold (C_t)</td>
<td>• Recommended for data analysis.</td>
</tr>
</tbody>
</table>
| Relative threshold \(C_{rt}\) | • \(Optional\) Use for data analysis.  
• Use to troubleshoot unexpected results.  
• Use to correct a variable baseline, which can be due to dried-down assays on the plate being reconstituted at different rates. |

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

Thermal protocols

The thermal protocol settings depend on:
• The real-time PCR instrument
• Whether the Master Mix requires fast or standard cycling mode based on its chemistry
• Whether the Master Mix contains UNG

The thermal protocols in “Set up and run the real-time PCR instrument” on page 15 are optimized for the TaqMan™ Fast Advanced Master Mix.

The following tables provide thermal protocols for other Master Mixes that are compatible with TaqMan™ Gene Expression Assays.

IMPORTANT! The cycling mode depends on the Master Mix that is used in the reaction. The cycling mode does not depend on a Standard or a Fast plate format.

Table 11  TaqMan™ Gene Expression Master Mix or TaqMan™ Universal Master Mix II, with UNG (any compatible instrument)

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time (standard cycling mode)</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNG incubation[1]</td>
<td>50°C</td>
<td>2 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Enzyme activation</td>
<td>95°C</td>
<td>10 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Denature</td>
<td>95°C</td>
<td>15 seconds</td>
<td>40</td>
</tr>
<tr>
<td>Anneal / Extend</td>
<td>60°C</td>
<td>1 minute</td>
<td></td>
</tr>
</tbody>
</table>

### Table 12  TaqMan™ Universal Master Mix II, no UNG (any compatible instrument)

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time (standard cycling mode)</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme activation</td>
<td>95°C</td>
<td>10 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Denature</td>
<td>95°C</td>
<td>15 seconds</td>
<td></td>
</tr>
<tr>
<td>Anneal / Extend</td>
<td>60°C</td>
<td>1 minute</td>
<td>40</td>
</tr>
</tbody>
</table>

### Table 13  TaqMan™ Fast Universal PCR Master Mix, no AmpErase™ UNG (StepOnePlus™, ViiA™ 7, or QuantStudio™ system)

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time (fast cycling mode)</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme activation</td>
<td>95°C</td>
<td>20 seconds</td>
<td>1</td>
</tr>
<tr>
<td>Denature</td>
<td>95°C</td>
<td>1 second</td>
<td></td>
</tr>
<tr>
<td>Anneal / Extend</td>
<td>60°C</td>
<td>20 seconds</td>
<td></td>
</tr>
</tbody>
</table>

### Table 14  TaqMan™ Fast Universal PCR Master Mix, no AmpErase™ UNG (7500 or 7500 Fast system)

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time (fast cycling mode)</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme activation</td>
<td>95°C</td>
<td>20 seconds</td>
<td>1</td>
</tr>
<tr>
<td>Denature</td>
<td>95°C</td>
<td>3 seconds</td>
<td></td>
</tr>
<tr>
<td>Anneal / Extend</td>
<td>60°C</td>
<td>30 seconds</td>
<td></td>
</tr>
</tbody>
</table>
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 uracil-N-glycosylase (UNG; also known as uracil-DNA glycosylase (UDG)) to degrade many contaminating carryover amplicons.

UNG enzymatic activity occurs during an initial incubation at 50°C. UNG is partially inactivated during the 95°C incubation step for template denaturation and polymerase activation. Because UNG is not completely deactivated during the 95°C incubation, it is important to keep the annealing temperatures greater than 55°C and to refrigerate PCR products at 2°C to 8°C in order to prevent amplicon degradation.

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.
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 adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's 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 necessary) 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.
**Biological hazard safety**

**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.

### Related documentation

<table>
<thead>
<tr>
<th></th>
<th></th>
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<tbody>
<tr>
<td><em>TaqMan™</em> Gene Expression Assays Quick Reference—96-well Standard (0.2-mL) Plates</td>
<td>4391139</td>
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<tr>
<td><em>TaqMan™</em> Gene Expression Assays Quick Reference—96-well Fast (0.1-mL) Plates</td>
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<tr>
<td><em>TaqMan™ PreAmp Master Mix User Guide</em></td>
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</tr>
<tr>
<td><em>TaqMan™ PreAmp Master Mix Quick Reference</em></td>
<td>4384556</td>
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<tr>
<td><em>Understanding Your Shipment</em></td>
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<td>For detailed information about the Assay Information File (AIF)</td>
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<td><em>QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems</em></td>
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<td><em>TaqMan™ Array Plates with RFID for use with QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems Quick Reference Guide</em></td>
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<td><em>StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation, Networking and Maintenance User Guide</em></td>
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Customer and technical support

Visit thermofisher.com/support for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - 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.