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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ThermoFisher SCIENTIFIC

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

The information in this guide is subject to change without notice.

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Revision	Date	Description
J	24 May 2019	• Added product information and procedures for TaqMan® Array Plates with RFID. • Added QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems.
H	15 May 2018	• Updated thermal cycling conditions for TaqMan Fast Advanced Master Mix. • Added option of Crt algorithm for troubleshooting. • Reorganized product information chapter. • Corrected troubleshooting for inhibitors in the real-time PCR reaction.
G	29 November 2017	• Updated information to obtain files for the plates. • Updated storage for the plates. • Added new instruments, Master Mixes, and other applicable products. • Added thermal cycling protocols for all compatible Master Mixes. • Updated guidelines for reverse transcription. • Removed content that is described in other resources; added references as appropriate. • Updated for general style, formatting, and branding.
F	September 2011	Baseline for this revision history.

Contents

1 Product information

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 28 for more information)

The following configurations are available.

Configuration	Description	Customizable
Fixed-content TaqMan® Array Plates	Preplated and predefined TaqMan® Gene Expression Assays that are manufactured and stocked in advance	No
Flexible-content TaqMan® Array Plates	Plates configured with a suggested selection of TaqMan® Gene Expression Assays, categorized by disease, pathway, or biological process	Preselected assays can be substituted with other predesigned assays that target TaqMan® Gene Expression Assays more applicable to experiment needs

Configuration	Description	Customizable
Custom TaqMan® Array Plates	Fully customizable plates	Allows the configuration of the TaqMan® Array Plates with any predesigned assays
TaqMan® Array Plates with RFID		

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 32).

For detailed information about TaqMan® Gene Expression Assays, see page 28.

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

Format	Cat. No. (Standard, 0.2-mL)	Cat. No. (Fast, 0.1-mL)	Number of assays + controls	Number of samples	Storage[1]
TaqMan® Array Plate 8	4413266	4413263	7+ 1	12	15–30°C[2]
TaqMan® Array Plate 16	4413264	4413261	15+ 1	6	
TaqMan® Array Plate 16 Plus[3]	4413265	4413262	12+ 4	6	
TaqMan® Array Plate 32	4391528	4413259	31+ 1	3	
TaqMan® Array Plate 32 Plus[3]	4391529	4413260	28+ 4	3	
TaqMan® Array Plate 48	4391526	4413257	47+ 1	2	
TaqMan® Array Plate 48 Plus[3]	4391527	4413258	44+ 4	2	
TaqMan® Array Plate 96	4391524	4413255	95+ 1	1	
TaqMan® Array Plate 96 Plus[3]	4391525	4413256	92+ 4	1	

[1] See packaging for expiration date.

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

[3] TaqMan® "Plus" plates only use *human* endogenous controls; controls for other species are not supported.

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**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Table 2 Recommended products for isolation of RNA

Item	Source
Kits for RNA isolation	thermofisher.com/rnaisolation

Table 3 Recommended products for preparation of cDNA

Item	Source
cDNA kit or cDNA Master Mix, one of the following:	
SuperScript™ IV VILO™ Master Mix	11756050
SuperScript™ IV VILO™ Master Mix with ezDNase™ Enzyme	11766050
High-Capacity cDNA Reverse Transcription Kit	4368813

Table 4 PCR Master Mixes

Item	Source
(Recommended) TaqMan® Fast Advanced Master Mix	4444556
TaqMan® Gene Expression Master Mix	4369016
TaqMan® Universal Master Mix II, with UNG	4440038
TaqMan® Universal Master Mix II, no UNG	4440047
TaqMan® Fast Universal PCR Master Mix, no AmpErase™ UNG	4352042

Table 5 Other materials and equipment required for the workflow

Item	Source
Real-time PCR instrument, one of the following:	
QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems	Contact your local sales office
QuantStudio™ 3 or 5 Real-Time PCR System	
QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 12K Flex Real-Time PCR System	
StepOnePlus™ Real-Time PCR System	
ViiA™ 7 Real-Time PCR System	
7500/7500 Fast Real-Time PCR System	
7900HT Fast Real-Time PCR System	

Item	Source
Software	
(Optional) Relative Quantification application	Available on the Connect platform
(Optional) ExpressionSuite™ Software	Available at **thermofisher.com/expressionsuite**
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 adapter for 96-well plates	MLS
Microcentrifuge	MLS
Vortex mixer	MLS
(Optional) Eppendorf™ MixMate™ (shaker)	Fisher Scientific™ 21-379-00
Pipettes	MLS
Tubes, plates, and other consumables	
Tubes, plates, and film	**thermofisher.com/plastics**
Aerosol-resistant barrier pipette tips	MLS
Disposable gloves	MLS
Reagents	
Nuclease-free Water	AM9930
RNase Inhibitor	N8080119
RNaseOUT™ Recombinant Ribonuclease Inhibitor	10777019
TURBO DNA-*free*™ Kit	AM1907
(Optional) TaqMan® PreAmp Master Mix	4391128
(Optional) TaqMan® PreAmp Master Mix Kit	4384267

Workflow

Start with cDNA templates from RNA samples (page 12)

▼

Combine cDNA and Master Mix (page 14)

▼

Prepare the TaqMan® Array Plate (page 14)

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

▼

Analyze the results (page 17)

2 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.

3 Perform real-time PCR

Procedural guidelines for performing real-time PCR

- Follow best-practices when preparing or performing PCR (see page 34).
- 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 6 96-well Standard (0.2-mL) Plate

Component	Volume			
	1 well	8 wells[1]	32 wells[1]	96 wells[1]
cDNA sample + nuclease-free water[2]	10 µL	90 µL	360 µL	1,080 µL
Master Mix (2X)[3]	10 µL	90 µL	360 µL	1,080 µL
Total volume	**20 µL**	**180 µL**	**720 µL**	**2,160 µL**

[1] Includes 12.5% overage.
[2] Ensure that the final cDNA concentration per well is 1–100 ng per 20-µL reaction.
[3] TaqMan® Fast Advanced Master Mix is recommended.

Table 7 96-well Fast (0.1-mL) Plate

Component	Volume			
	1 well	8 wells[1]	32 wells[1]	96 wells[1]
cDNA sample + nuclease-free water[2]	5 µL	45 µL	180 µL	540 µL
Master Mix (2X)[3]	5 µL	45 µL	180 µL	540 µL
Total volume	**10 µL**	**90 µL**	**360 µL**	**1,080 µL**

[1] Includes 12.5% overage.
[2] Ensure that the final cDNA concentration per well is 5–50 ng per 10-µL reaction.
[3] TaqMan® Fast Advanced Master Mix is recommended.

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. Centrifuge 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 31 for instructions to import setup files.

   Note: The setup files are instrument-specific. See "Instrument software and setup files" on page 31 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 32 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)

Step	Temperature	Time	Cycles
UNG incubation[1]	50°C	2 minutes	1
Enzyme activation	95°C	20 seconds[2]	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

[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)

Step	Temperature	Time	Cycles
UNG incubation[1]	50°C	2 minutes	1
Enzyme activation	95°C	20 seconds[2]	1
Denature	95°C	3 seconds	40
Anneal / Extend	60°C	30 seconds	

[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 ($\Delta\Delta C_t$) method to analyze results.

The general guidelines for analysis include:

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

Perform additional data analysis using any of the following software:

Software	Resource
Relative Quantification application	**thermofisher.com/connect**
ExpressionSuite™ Software[1]	**thermofisher.com/expressionsuite**

[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**.

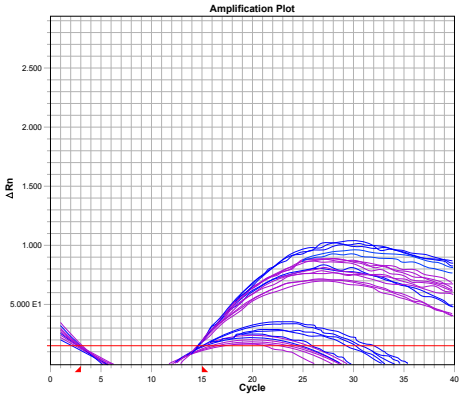
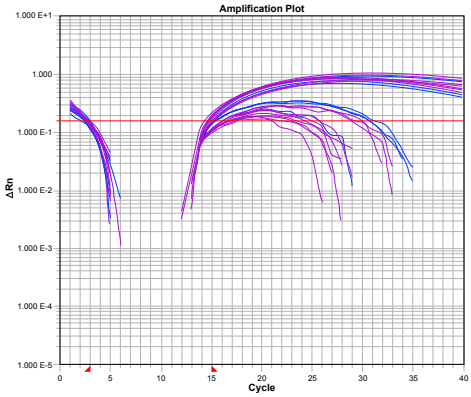
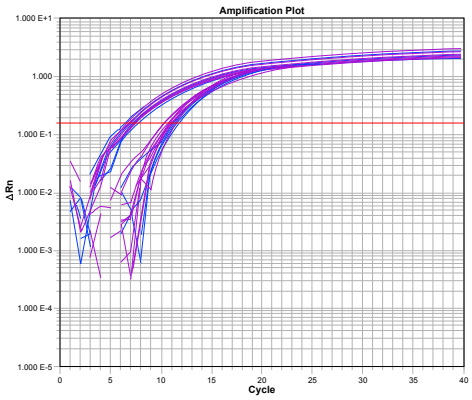
Data can be analyzed using the relative threshold algorithm (C_{rt}).

See "Algorithms for data analysis" on page 32.

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_t and C_{rt}.

Troubleshooting

Observation	Possible cause	Recommended action
Amplification curve shows abnormal plot and/or low ΔRn values Linear view:  Log view:  	The baseline was set improperly (some samples have C_t values lower than the baseline stop value).	See your real-time PCR system user guide for procedures on setting the baseline. Switch from manual to automatic baselining, or move the baseline stop value to a lower C_t (2 cycles before the amplification curve crosses the threshold). Corrected log view: 
	An amplification signal is detected in the early cycles (no baseline can be set because the signal is detected too early).	Dilute the sample to increase the C_t value.
The Multicomponent Plot shows low ROX™ dye (passive reference dye)	Little or no Master Mix is present due to inaccurate pipetting.	Follow accurate pipetting practices.

Observation	Possible cause	Recommended action
Amplification curve shows no amplification of the sample (C_t=40) across all assays or in an unusually large number of assays	One or more of the reaction components was not added.	Ensure that the cDNA and the Master Mix were added to the reaction plate. The passive reference fails if the Master Mix is missing.
	Incorrect dyes were selected for each target.	Check the dyes selected for each target, then reanalyze the data.
	The annealing temperature was too high for the primers and/or probe.	Ensure that the correct annealing and extension temperatures are set.
		Ensure that the real-time PCR instrument is calibrated and maintained regularly.
	Inappropriate reaction conditions were used.	Troubleshoot the real-time PCR optimization.
		Ensure that the properties and the thermal protocol are correct.
	The template is degraded.	• Determine the quality of the template. • Rerun the assay with fresh template. • Use RNase-free reagents. • Use an RNase inhibitor.
	Inhibitors are present in the reaction.	Ensure the presence of an inhibitor: 1. Create a serial dilution of your sample. 2. Run the serial dilution with an expressing assay (for example, an endogenous control). If an inhibitor is present, high concentrations yield higher-than-expected C_t values. (High concentration means more inhibition because the sample is not diluted.) 3. Rerun the assay with purified template.
	The baseline and/or threshold was improperly set.	See your real-time PCR system user guide for procedures on setting the baseline and threshold: • Switch from an automatic baseline to a manual baseline, or from a manual baseline to an automatic baseline. • Lower the threshold value to within the appropriate range.
	The reverse transcription failed.	• Check the RNA integrity and concentration. • Check for RNase activity. • Follow the recommended thermal profile. • Repeat the reverse transcription using new reagents.

Observation	Possible cause	Recommended action
Amplification curve shows samples targeted by the same assay that have differently shaped curves	The baseline was set improperly.	See your real-time PCR system user guide for procedures on setting the baseline: • Switch from an automatic baseline to a manual baseline, or from a manual baseline to an automatic baseline. • Increase the upper or lower value of the baseline range.
	The sample quality was poor.	1. Perform a quality check on the sample. 2. If needed, re-extract the sample.
	There were different concentrations caused by imprecise pipetting.	Follow accurate pipetting practices.
	The reagents or equipment are contaminated.	Be sure that your workspace and equipment are cleaned properly.

Observation	Possible cause	Recommended action
Amplification curve shows no amplification of the sample (C_t=40) in the target assay	The gene is not expressed in the tested sample.	• Ensure that the gene is expressed in the sample type or tissue type. Go to **ncbi.nlm.nih.gov/unigene**. • Confirm the results. – Rerun the sample using the same assay. – Rerun the experiment using more sample. Avoid preparing PCR reaction mixes with more than 20% reverse transcription reaction. – Run the experiment using an alternative assay, if available, that detects a different transcript or more than one transcript from the same gene. **Note:** If the recommended actions do not resolve the problem, the result may be correct.
	The sample does not have enough copies of the target RNA.	Confirm the results. • Rerun the sample using the same assay. • Rerun the assay using more sample. Avoid PCR reaction mix with more than 20% from the reverse transcription reaction. **Note:** If the recommended actions do not resolve the problem, the result may be correct.
	One or more of the reaction components was not added.	Check your pipetting equipment and/or technique.
	Incorrect dyes were selected for each target.	Check the dyes selected for each target, then reanalyze the data.
Decrease in ROX™ dye fluorescence (passive reference dye)	There was precipitation in the buffers.	Mix the Master Mix thoroughly to produce a homogenous solution.
	The reagents are degraded.	Ensure that the kits and reagents have been stored according to the instructions on the packaging and that they have not expired.
There was a simultaneous increase in fluorescence from both the passive reference dye (ROX™ dye) and the reporter dyes	The sample evaporated.	Check the seal of the adhesive film for leaks.
The multicomponent signal for ROX™ dye is not flat	Incorrect dyes were selected for each target.	Check the dyes selected for each target, then reanalyze the data.

Observation	Possible cause	Recommended action
The Rn in the Rn vs Cycle plot is very high	The ROX™ dye was not set as the passive reference.	Set ROX™ dye as the passive reference, then reanalyze the data.
The no template control (NTC) shows amplification	The reagents are contaminated with gDNA, amplicon, or plasmid clones.	• Rerun the assay using new reagents. • Ensure that your workspace and equipment are cleaned properly. • Use a Master Mix that contains UNG. • Run no-RT controls to rule out genomic DNA contamination. • Treat the sample with DNase.
The endogenous control C_t values vary or they do not normalize the sample well	The endogenous control is not consistently expressed across the samples.	Ensure that the endogenous control is consistently expressed in your sample type. See "Endogenous controls" on page 28.
	The sample concentrations vary.	Quantitate and normalize the PCR samples.
	Pipetting was inaccurate.	• Check the calibration of the pipettes. • Pipet at least 5 µL of sample to prepare the reaction mix.
There is a high standard deviation in the replicates, inconsistent data, or a variable C_t	The reagents were not mixed properly.	• Increase the length of time that you mix the reagents. • Verify your mixing process by running a replicate assay.
	Pipetting was inaccurate.	• Check the calibration of the pipettes. • Pipette at least 5 µL of sample to prepare the reaction mix.
	The threshold was not set correctly.	Set the threshold above the noise level and where the replicates are tightest. See your real-time PCR system user documentation.
	There was a low concentration of the target of interest.	Rerun the assay using more cDNA template.
The C_t value is lower than expected	Genomic DNA (gDNA) contamination occurred.	• Run a no-RT control to confirm that there was gDNA contamination. • Use DNase to ensure minimal gDNA contamination of the RNA.
	Too much cDNA template was added.	• Reduce the amount of cDNA template. • Quantitate the RNA before the RT reaction, then adjust the concentration of cDNA from the RT reaction that is added to the real–time PCR reaction.
	The cDNA template or the amplicon is contaminated.	Follow established PCR good laboratory practices.

Observation	Possible cause	Recommended action
Amplification occurs in the no-RT controls	Genomic DNA (gDNA) contamination occurred.	• Improve sample extraction methods to eliminate gDNA. See Chapter 2, "Guidelines for preparation of cDNA". • Use DNase to ensure minimal gDNA contamination of the RNA.
	The cDNA template or amplicon is contaminated.	Follow established PCR good laboratory practices.
There was a shifting Rn value during the early cycles of the PCR (cycles 0 to 5)	Fluorescence did not stabilize to the buffer conditions of the reaction mix. **Note:** This condition does not affect PCR or the final results.	• Reset the lower value of the baseline range. • Use an automatic baseline. • Use the relative threshold algorithm (C_{rt}). See *Introduction to Gene Expression Getting Started Guide* (Pub. No. 4454239).
There was a small ΔRn	The PCR efficiency was poor.	Ensure that the reagents were used at the correct concentration.
	The quantity of the cDNA is low (a low copy number of the target).	Increase the quantity of the cDNA.
There is a noisy signal above the threshold	The sample evaporated.	Check the seal of the adhesive film for leaks.
	The well is empty because of inaccurate pipetting.	• Check the calibration of the pipettes. • Pipet at least 5 µL of sample.
	The well is assigned a sample or target in the plate document or experiment, but the well is empty.	• Be sure that your plate document or experiment is set up correctly. • Exclude the well and reanalyze the data.
The baseline is variable	The dried-down assays on the TaqMan® Array Plate were reconstituted at different rates, causing a dip in the early cycles of the baseline.	Use the relative threshold algorithm (C_{rt}). C_{rt} may correct for a variable baseline.
		Use the Relative Quantification application, available on Connect. The Relative Quantification application uses C_{rt} if your instrument software does not have the relative threshold algorithm.

B

Detailed procedures for TaqMan® Array Plates with RFID

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 32.

Step	Temperature	Time	Cycles
UNG Incubation	50°C	2 minutes	1
Enzyme Activation	95°C	20 seconds	1
Denature	95°C	1 second	40
Anneal/Extend	60°C	20 seconds	

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.

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 36 and "Biological hazard safety" on page 37.

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 27).

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**.

C

Supplemental information

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. CO01971 0612), 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™) 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.

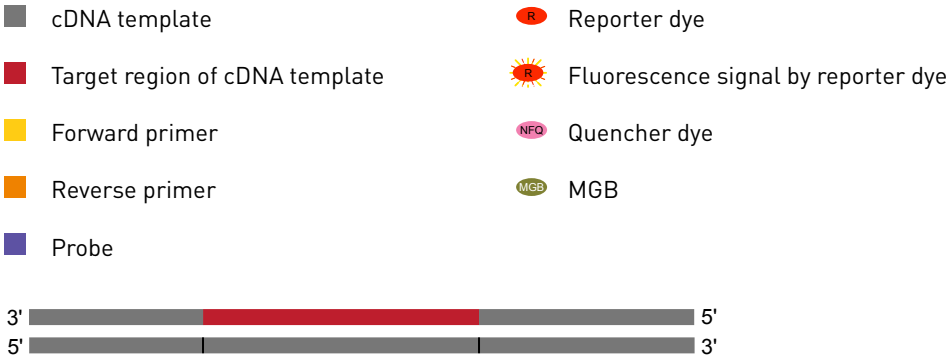


Figure 1 cDNA synthesis product

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.

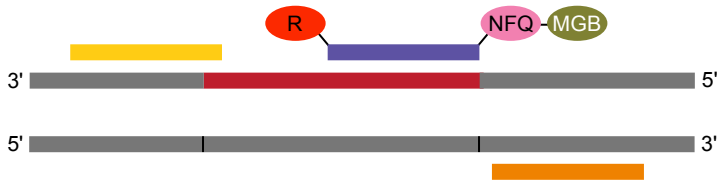


Figure 2 Annealing of probes and primers to cDNA strands

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.

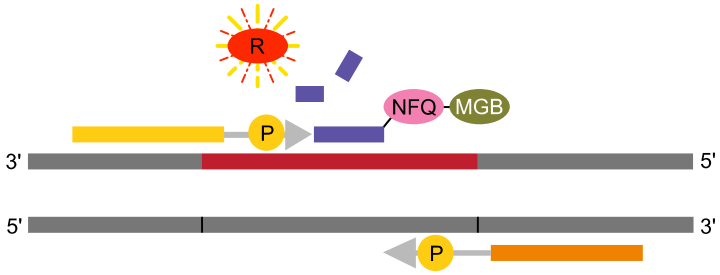


Figure 3 Initial polymerization and cleavage of reporter dye

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.

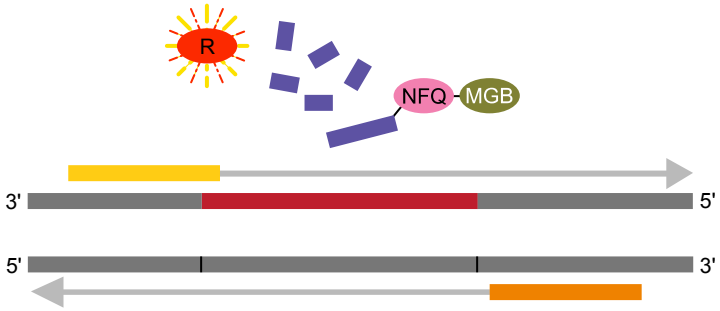


Figure 4 Completion of polymerization

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

Instrument software	Setup file
QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems	`ordernumber_7500_2.0_FAST.txt`
QuantStudio™ 3 or 5 Real-Time PCR System	
QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 12K Flex Real-Time PCR System	
ViiA™ 7 Real-Time PCR System	
StepOnePlus™ Real-Time PCR System	`ordernumber_StepOne_2.1.txt`
7500/7500 Fast Real-Time PCR System (v2.0)	`ordernumber_7500_2.0_FAST.txt`
7500/7500 Fast Real-Time PCR System (v1.0)	`ordernumber_7300_7500_SDS.txt`

Import the setup files

Real-time PCR instrument	Navigation
QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems	In the menu bar during setup, select **File ▸ Import Plate Setup**.
QuantStudio™ 3 or 5 Real-Time PCR System	
QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 12K Flex Real-Time PCR System	
ViiA™ 7 Real-Time PCR System	
StepOnePlus™ Real-Time PCR System	In the menu bar, select **File ▸ Import**.
7500/7500 Fast Real-Time PCR System (v2.0)	In the menu bar, select **Plate Setup ▸ File ▸ Import**.
7500/7500 Fast Real-Time PCR System (v1.0)	In the menu bar, select **File ▸ Import Sample Setup**.

Algorithms for data analysis

Table 10 Algorithm recommendations for TaqMan® Array Plates

Algorithm	Recommendation
Threshold (C_t)	• Recommended for data analysis.
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)

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

[1] For optimal UNG activity.

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

Step	Temperature	Time (standard cycling mode)	Cycles
Enzyme activation	95°C	10 minutes	1
Denature	95°C	15 seconds	40
Anneal / Extend	60°C	1 minute	

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

Step	Temperature	Time (fast cycling mode)	Cycles
Enzyme activation	95°C	20 seconds	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

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

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

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.

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 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.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Pub. No.
TaqMan® Gene Expression Assays Quick Reference—96-well Standard (0.2-mL) Plates	4391139
TaqMan® Gene Expression Assays Quick Reference—96-well Fast (0.1-mL) Plates	4427562
Introduction to Gene Expression Getting Started Guide	4454239
TaqMan® PreAmp Master Mix User Guide	4384557
TaqMan® PreAmp Master Mix Quick Reference	4384556
Understanding Your Shipment For detailed information about the Assay Information File (AIF)	MAN0017153
QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems	
QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems User Guide	MAN0018045
TaqMan® Array Plates with RFID for use with QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems Quick Reference Guide	MAN0018436
QuantStudio™ 3 or 5 Real-Time PCR System	
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
QuantStudio™ Design and Analysis Desktop Software User Guide	MAN0010408
QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System	
QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide	4489821
QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Getting Started Guide	4489822
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
StepOnePlus™ Real-Time PCR System	
StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation, Networking and Maintenance User Guide	4376782
Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C_t Experiments Getting Started Guide	4376785

Document	Pub. No.
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
7500/7500 Fast Real-Time PCR System	
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4347828
Applied Biosystems™ 7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Standard Curve and Comparative C_t Experiments	4387783

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- Worldwide contact telephone numbers
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  - 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**.