

TaqPath[™] qPCR Master Mix, CG

USER GUIDE

For two-step RT-PCR in gene expression experiments or
quantitative analysis

Catalog Numbers A15297, A16245, A16247, A16248, A15298

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Manufacturer: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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Revision	Date	Description
2.00	28 February 2018	• Change 50°C UNG incubation to optional • Add TaqMan® Gene Expression Assays • Update licensing, trademarks, general style and format
1.0	10 September 2013	User Guide to accompany new product introduction

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Product information

Product description

The TaqPath™ qPCR Master Mix, CG enables PCR in any gene expression experiment or quantitative analysis, including:

- Differential gene expression analysis
- MicroRNA quantification
- Microarray validation
- Pathogen detection
- Viral load quantification

TaqPath™ qPCR Master Mix, CG can be used with any DNA target, including complementary DNA (cDNA) or genomic DNA (gDNA). It can be used in the second step of a two-step RT-PCR protocol for RNA quantitation experiments. A cDNA template can be generated from RNA using one of our reverse transcription kits prior to PCR. TaqPath™ qPCR Master Mix, CG is designed to accommodate duplex reactions (target + endogenous/exogenous control). See [thermofisher.com/multiplexqpcr](https://www.thermofisher.com/multiplexqpcr) for information about multiplex reactions and compatible master mixes.

TaqPath™ qPCR Master Mix, CG is supplied at a 2X concentration and contains:

- Fast DNA Polymerase
- Uracil N-Glycosylase (UNG)
- dNTPs with dUTP
- ROX™ dye (passive reference)
- Optimized buffer components

See “Master mix components” on page 13 for more information about the master mix components.

TaqPath™ qPCR Master Mix, CG is optimized for use with primers and TaqMan® probes designed according to our guidelines.



Contents and storage

Table 2 TaqPath™ qPCR Master Mix, CG (2X)

Cat. No.	Number of reactions	Amount	Storage ^[1]
A15297	500	1 × 5-mL	2–8°C
A16245	1000	2 × 5-mL	
A16247	2500	5 × 5-mL	
A16248	5000	10 × 5-mL	
A15298	5000	1 × 50-mL	

^[1] See packaging for expiration date.

Required materials not supplied

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

Table 3 Instrument, software, equipment, plates and accessories, and consumables

Item	Source
Instrument, one of the following:	
QuantStudio™ Dx Real-Time PCR Instrument	Contact your local sales office.
7500 Fast Dx Real-Time PCR Instrument	
Equipment	
Centrifuge with plate adapter	MLS
Microcentrifuge	MLS
Thermal cycler, or heat block or water bath	MLS
Adjustable pipettors	MLS
Laboratory mixer (vortex or equivalent)	MLS
Tubes, plates, and other consumables	
Optical reaction plates and adhesive covers	thermofisher.com/plastics
Pipette tips	thermofisher.com/pipettetips
Disposable gloves	MLS

**Table 4** Kits and reagents for reverse transcription

Item	Source
Reverse transcription products	thermofisher.com/cdna
TE, pH 8.0	AM9849
(Optional) RNase Inhibitor	N8080119 AM2684 (Cloned; 40 U/μL)
Nuclease-free Water (not DEPC-Treated)	AM9930

Table 5 Assays

Item	Source
TaqMan® Gene Expression Assays	thermofisher.com/taqmangeneexpression
Custom TaqMan® Gene Expression Assays	thermofisher.com/taqmancustomgeneexpression
Custom TaqMan® probes and primers ^[1]	thermofisher.com/customprimersprobes

^[1] Synthesized to your sequence and choice of quencher and reporter dyes.

Table 6 Kits and reagents for RNA isolation

Item	Source
RNA isolation products	thermofisher.com/rnaisolation
Supporting reagents	thermofisher.com/rnaisolationreagents

Workflow

Prepare the PCR Reaction Mix (page 9)



Prepare the PCR reaction plate (page 9)



Set up and run the real-time PCR instrument (page 10)



Methods

Procedural guidelines

Guidelines for reverse transcription

Perform reverse transcription to obtain cDNA from RNA samples. Omit the reverse transcription step and proceed directly to real-time PCR amplification if using gDNA samples.

- See Table 6 on page 7 for kits and reagents for RNA isolation.
- See Table 4 on page 7 for kits and reagents for reverse transcription.
- For optimal reverse transcription, input RNA should be:
 - Free of inhibitors of reverse transcription (RT) and PCR
 - Dissolved in PCR-compatible buffer
 - Free of RNase activity
 - Nondenatured total RNA
- For the input RNA amount, follow the recommendations provided by the reverse transcription kit.
- Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes, then include 10% overage, unless otherwise indicated.

Guidelines for PCR reactions

- Store TaqMan® Assays frozen and away from light until use. Excessive exposure to light may affect the fluorescent probes.
- We recommend three replicates reactions for each assay.
- Multiple assays can be run on one reaction plate. Include no-template controls (NTCs) for each assay.
- Review “Best practices for PCR and RT-PCR experiments” on page 16.

Before you begin

1. Thaw the TaqPath™ qPCR Master Mix, CG on ice, then mix thoroughly but gently.
2. Thaw TaqMan® Assays on ice, then vortex and briefly centrifuge to resuspend.
3. Thaw samples on ice, then vortex and briefly centrifuge to resuspend.



Prepare the PCR Reaction Mix

- Combine the following components for the number of reactions required, plus 10% overage.

Component	Volume per reaction			Final concentration
	384-well plate	96-well Fast (0.1-mL) Plate	96-well Standard (0.2-mL) Plate	
TaqPath™ qPCR Master Mix, CG	5.0 µL	10.0 µL	25.0 µL	1X
TaqMan® Gene Expression Assay	≤ 3.0 µL	≤ 8.0 µL	≤ 20.0 µL	1X
Nuclease-free Water	Variable	Variable	Variable	—
Total volume per reaction	8.0 µL	18.0 µL	45.0 µL	—

- Vortex briefly to mix.
- Centrifuge briefly to bring the PCR Reaction Mix to the bottom of the tube and eliminate air bubbles.

Prepare the PCR reaction plate

- Combine the PCR Reaction Mix and cDNA in an optical reaction plate.

Component	Volume per reaction		
	384-well plate	96-well Fast (0.1-mL) Plate	96-well Standard (0.2-mL) Plate
PCR Reaction Mix	8.0 µL	18.0 µL	45.0 µL
cDNA template or Nuclease-free Water (for NTC reactions)	2.0 µL	2.0 µL	5.0 µL
Total volume per reaction	10.0 µL	20.0 µL	50.0 µL

- Transfer the appropriate volume of PCR Reaction Mix to each well of an optical reaction plate.
- Seal the plate with optical adhesive film, then centrifuge briefly to bring the PCR Reaction mix to the bottom of the well and eliminate air bubbles.
- Remove the optical adhesive film.



- d. Add cDNA template (1 pg to 100 ng in Nuclease-free Water), or Nuclease-free Water for NTC, to each well.
2. Seal the reaction plate with optical adhesive film, then invert the plate 3–5 times, making sure that the contents of the wells are moving back and forth between the seal and the bottom of the wells to ensure proper mixing.
Note: Inverting the plate gives more uniform mixing across the reaction plate than vortexing.
3. Centrifuge briefly to bring the contents to the bottom of the well and eliminate air bubbles.
4. Apply a compression pad to the plate, if required by your instrument.

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.

1. Select the cycling mode.

Cycling mode	Reaction plate format
Standard	384-well, 96-well Fast (0.1-mL), 96-well Standard (0.2-mL)
Fast ^[1]	384-well, 96-well Fast (0.1-mL)

^[1] Not recommended for use with 96-well Standard (0.2-mL) Plates due to thermal transfer limitations associated with larger reaction volume.

2. Set up the thermal protocol for your instrument and cycling mode.

Table 7 Standard thermal-cycling profile

	(Optional) UNG incubation ^[1]	Polymerase activation ^[2,3]	PCR (40 cycles)	
Real-time PCR instrument	Hold 50°C	Hold 95°C	Denature 95°C	Anneal/extend 60°C
QuantStudio™ Dx Real-Time PCR Instrument	2 minutes	20 seconds	15 seconds	1 minute

^[1] Heat-labile UNG is active during the reaction setup and does not require a 50°C incubation for UNG activity. Product performance was validated with the optional 50°C UNG incubation step.

^[2] The polymerase activation/initial denaturation can be increased up to 10 minutes for templates that are difficult to denature.

^[3] Heat-labile UNG is completely inactivated during the first ramp to the 95°C hold step.



Table 8 Fast thermal-cycling profile

Real-time PCR instrument	(Optional) UNG incubation ^[1]	Polymerase activation ^[2,3]	PCR (40 cycles)	
	Hold 50°C	Hold 95°C	Denature 95°C	Anneal/extend 60°C
QuantStudio™ Dx Real-Time PCR Instrument	2 minutes	20 seconds	1 second	20 seconds
7500 Fast Dx Real-Time PCR Instrument	2 minutes	20 seconds	3 seconds	30 seconds

^[1] Heat-labile UNG is active during the reaction setup and does not require a 50°C incubation for UNG activity. Product performance was validated with the optional 50°C UNG incubation step.

^[2] The polymerase activation/initial denaturation can be increased up to 10 minutes for templates that are difficult to denature.

^[3] Heat-labile UNG is completely inactivated during the first ramp to the 95°C hold step.

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

Reaction plate format	Reaction volume (µL)
384-well	10.0
96-well Fast (0.1-mL)	20.0
96-well Standard (0.2-mL)	50.0

4. Load the plate into the real-time PCR instrument, then start the run.

Guidelines for data analysis

Data analysis varies depending on your real-time PCR instrument. See the appropriate instrument documentation for more information.

1. View the amplification plots for the reactions.
2. Use auto baseline and auto threshold settings, or set the baseline and threshold values to determine the threshold cycles (C_t) for the amplification curves.

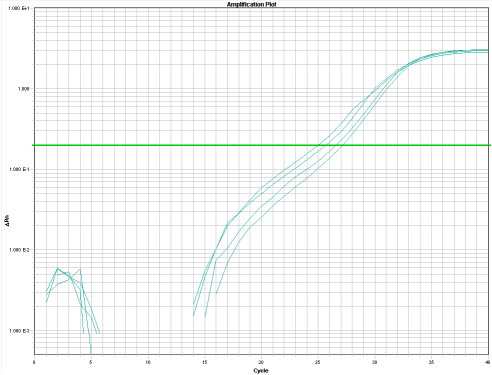
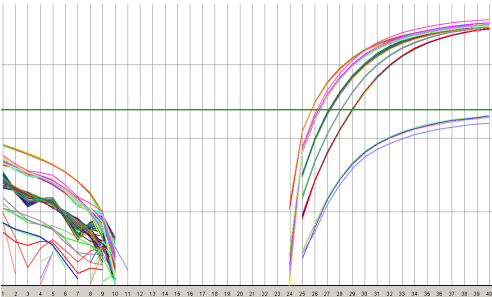
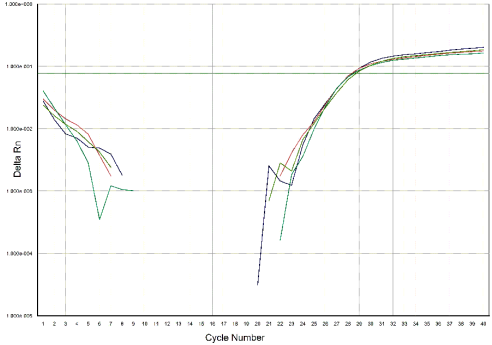
About baseline and threshold values

You can use the real-time PCR system software to set the baseline and threshold values for the amplification plot, either automatically or manually.

- The *baseline* refers to the initial cycles of PCR in which there is slight change in fluorescence signal.
- The intersection of the *threshold* with the amplification plot defines the C_t in real-time PCR assays. The threshold is set above the background signal and within the exponential growth phase of the amplification curve.



Troubleshooting

Observation	Possible cause	Recommended action
The amplification plot displays S-shaped curves 	The qPCR mix was not thoroughly combined.	When preparing the qPCR mix (page 9–page 9), be sure to invert the reaction plate 3 to 5 times to mix before centrifuging.
The amplification plot is truncated 	The baseline was set too high.	When analyzing the data (page 11), manually reset the baseline, or use the automatic baseline function.
The C_t is low, but merges with the background signal [noise] 	When automatic baseline is used, the software raises the threshold bar to avoid the elevated baseline.	When analyzing the data (page 11), change analysis setting to allow manual adjustment of the threshold, then manually adjust the threshold bar.



Supplemental information

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■ TaqMan® Gene Expression Assays chemistry overview	13
■ Best practices for PCR and RT-PCR experiments	16

Master mix components

Heat-labile Uracil N-Glycosylase	Heat-labile Uracil N-Glycosylase (UNG) treatment can prevent the reamplification of carryover PCR products by removing any uracil incorporated into single- or double-stranded amplicons . UNG prevents reamplification of carryover PCR products in an assay if all previous PCR for that assay was performed using a dUTP-containing master mix. See “Use UNG to prevent false-positive amplification” on page 16 for more information about heat-labile UNG.
dUTP	This master mix includes dUTP to enable uracil-N-glycosylase (UNG) activity and maintain optimal PCR results.
ROX™ dye	The ROX™ dye acts as a passive reference (absorption 575 nm; emission 600 nm) to provide an internal reference for normalizing the reporter-dye signal during data analysis for multiplex reactions. Normalization corrects for fluorescence fluctuations due to changes in concentration or volume.

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.
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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.

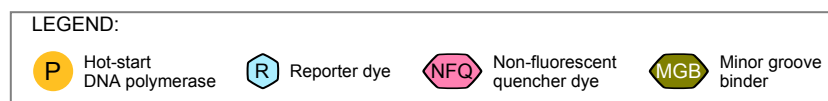


Figure 1 cDNA synthesis product

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.

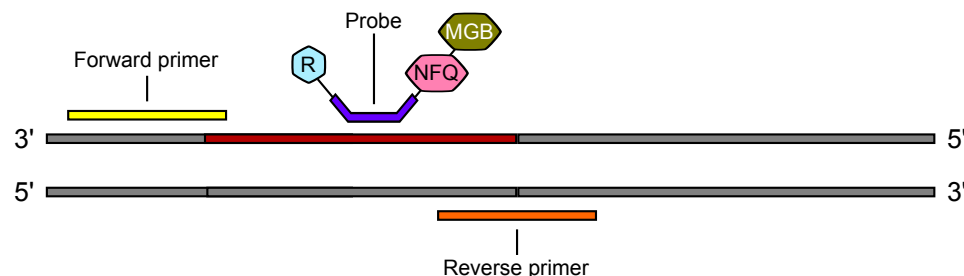


Figure 2 Annealing of probes and primers to cDNA strands

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.

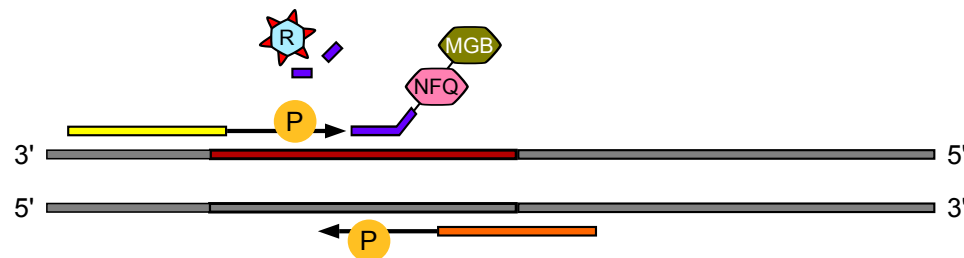


Figure 3 Initial polymerization and cleavage of reporter dye

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.

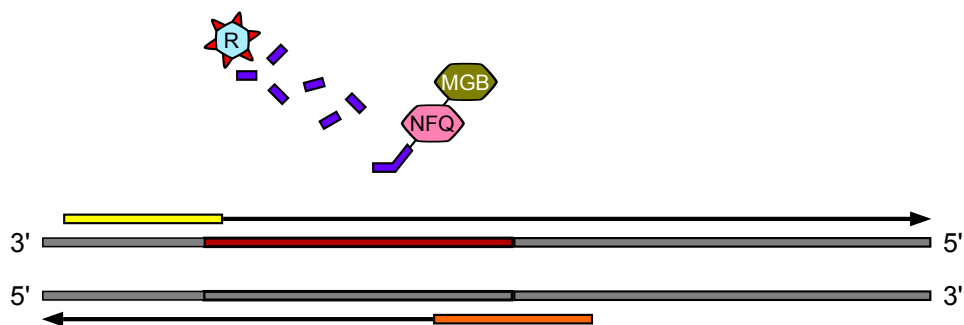


Figure 4 Completion of polymerization

Best practices for PCR and RT-PCR experiments

Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-PCR amplification:

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

Use UNG to prevent false-positive amplification

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

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

To ensure the desired UNG activity:

- Use PCR components and thermal cycling conditions as specified.

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

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

Detect fluorescent contaminants

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

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



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, etc). 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:
www.cdc.gov/biosafety/publications/bmbl5/BMBL.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.
Product documentation	
<i>Custom TaqMan[®] Assays Design and Ordering Guide</i>	4367671
<i>TaqMan[®] Gene Expression Assays User Guide—single-tube assays</i>	4333458
Instrument documentation	
<i>Applied Biosystems[™] QuantStudio[™] Dx Real-Time PCR Instrument User Guide</i>	4482540
<i>Applied Biosystems[™] 7500 Fast Dx Real-Time PCR Instrument Reference Guide</i>	4406991

Customer and technical support

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

- Worldwide contact telephone numbers
- Product support, including:
 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support
- Product documentation, including:
 - User guides, manuals, and protocols
 - 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

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