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TaqMan™ Roundup Ready™ Soya Quantification Kit user guide

Real-time PCR quantification of Roundup Ready[™] soya DNA in food and feed samples

for use with:

Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument Applied Biosystems™ 7500 Fast Real-Time PCR Instrument

Catalog Number 4466335

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Revision E







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Revision history: MAN0013477 D.0 (English)

Revision	Date	Description	
E	1 October 2024	The recommendation to include a 10% overage has been removed from the protocol for setting up the components for PCR reactions.	
D.0	19 January 2024	 The manufacturer address was updated. The trademark statement was updated. The storage temperature for the General Master Mix was updated. A note was added for real-time PCR instrument dye calibration. The KingFisher™ Flex Purification System with 96 Deep-Well Head was added. References to RapidFinder™ Analysis Software and RapidFinder™ Express Software were removed. 	
C.0	16 July 2021	 Troubleshooting was added. ISO certification was added. QuantStudio™ 5 Real-Time PCR Instrument with Thermo Scientific™ RapidFinder™ Analysis Software v1.2 or later was added. 	
B.0	27 July 2020	 The plate layout example was updated to include duplicate unknown sample reactions in Set up the PCR reactions. The MagMAX™ instrument was replaced by the KingFisher™ mL Magnetic Particle Processor. A footnote was added to the thermal cycler settings table in Set up and run the real-time PCR instrument. The manufacturing address and limited product warranty information were added. 	
A.0	10 October 2015	New document converted from Imegen document for the TaqMan™ Roundup Ready™ Soya Quantification Kit.	

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

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Product information

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IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The Thermo Scientific™ TaqMan™ Roundup Ready™ Soya Quantification Kit enables relative quantification of as little as 0.01% of Roundup Ready™ transgenic soybeans with respect to total soya in a sample.

The limit of quantification is 20 copies of DNA, and the detection limit of the PCR technique is 3 copies each of Roundup Ready™ and soya DNA.

The TaqMan™ Roundup Ready™ Soya Quantification Kit includes:

- Primers and TaqMan[™] probes for real-time PCR detection of:
 - The transgenic event characteristic of the soybean line GTS-40-3-2 (Roundup Ready™ soybean)
 - The endogenous soy gene, lectin
- Roundup Ready™ Soya Standard, a plasmid DNA quantitation standard containing both GTS-40-3-2 and lectin targets
- DNA polymerase enzyme and other buffer components necessary for real-time PCR

Principle of the relative quantification procedure

- 1. Two real-time PCR series are performed:
 - One detects the transgenic event characteristic of the soybean line GTS-40-3-2 (Roundup Ready™ soybean).
 - One detects the endogenous soy gene, lectin.
 - Each PCR series includes a dilution series of the Roundup Ready[™] Soya Standard in addition to the unknown samples and controls.
- 2. For each sample, the GTS-40-3-2 and lectin targets are quantified relative to the Roundup Ready™ Soya Standard.
- 3. The percentage of GTS-40-3-2 target with respect to lectin target is then calculated for that sample.

Kit contents and storage

Table 1 TaqMan™ Roundup Ready™ Soya Quantification Kit (Cat. No. 4466335)

Component	Amount (50 reactions)	Storage ^[1]
RR Soybean Master Mix (green disc)	360 μL	–20°C
Soya Master Mix (purple disc)	360 μL	–20°C
General Master Mix (white disc)	2 × 600 μL	-20°C upon receipt. 2-8°C after initial use. Store protected from light.
RR Soya Standard (green cap)	6 × 50 μL	–20°C

^[1] See the expiration date on the box.

Materials required but not provided

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

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

Item	Source	
Real-time PCR instrument, one of the following:		
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System	Contact your local microbiology sales representative	
Applied Biosystems™ 7500 Fast Real-Time PCR System		

(continued)

Item	Source				
Recommended equipment for automated DNA isolation					
KingFisher™ Flex Purification System with 96 Deep-Well Head	A32681				
Other equipment					
Adjustable micropipettors (10 μL, 20 μL, 200 μL)					
Benchtop microcentrifuge with adaptors for PCR plates and/or tubes	Available through the Thermo Fisher Microbiology ordering process. See thermofisher.com/plastics for more information.				
Laboratory mixer (Vortex mixer or equivalent)					
Optical reaction plates and covers, or optical PCR	tubes and caps				
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL	4346907				
MicroAmp™ Optical Adhesive Film, 100 covers	4311971				
MicroAmp™ Fast 8-Tube Strip, 0.1 mL (See below for caps.)	4358293				
MicroAmp™ Optical 8-Cap Strips	4323032				
Other plastics and consumables					
Aerosol-resistant pipette tips	Available through the Thermo Fisher Microbiology				
1.5-mL nuclease-free microcentrifuge tubes	ordering process. See thermofisher.com/plastics for more information.				
Powder-free disposable gloves					
Reagents					
Nuclease-free water (not DEPC-Treated)	AM9938				
Recommended kits for DNA isolation, one of the following:					
GMO Extraction Kit	4466336				
For high-throughput isolation:	101101				
Lysis Buffer 1 + RNase GMO Extraction Kit	A24401				
PrepSEQ™ Nucleic Acid Extraction Kit	4428176, 4480466				

Methods



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Input DNA requirements

- Prepare the DNA sample with a method that allows processing of 10-20 g of food sample.
 - For low-throughput, manual processing, use the GMO Extraction Kit.
 - For automated processing, it is recommended to use the Lysis Buffer 1 + RNase GMO
 Extraction Kit and the PrepSEQ™ Nucleic Acid Extraction Kit with the KingFisher™ Flex
 Purification System with 96 Deep-Well Head.
- Prepare at least one mock-purified sample as a negative extraction control, processed with the same DNA isolation method that is used for test samples.
- Dilute the final DNA sample to 10–25 ng/µL for the PCR.

Determine the number of reactions, then thaw the reagents

- 1. For each PCR series, plan to include the following reactions (see Figure 2):
 - Duplicates for each unknown sample
 - Freshly prepared dilution series of the Roundup Ready[™] Soya Standard (5 dilutions)
 - Negative extraction control (mock-purified samples)
 - No-template control reactions (NTC); use nuclease-free water in place of sample DNA
- 2. Thaw all reagents, vortex to mix thoroughly, then place on ice.

Prepare a dilution series of the Roundup Ready[™] Soya Standard

Prepare 1:10 serial dilutions of the Roundup Ready™ Soya Standard in nuclease-free water, as described in the following figure.

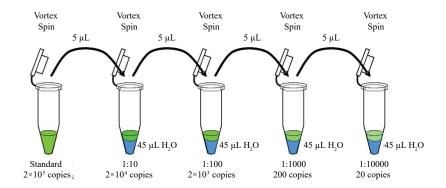


Figure 1 Serial dilution of the Roundup Ready™ Soya Standard The 1:10 serial dilutions are used to prepare standard curves for Roundup Ready™ Soybean and lectin. The number of copies is per 5 μL, or per PCR reaction.

Set up the PCR reactions

1. Prepare a reaction mix for each PCR series, for the number of reactions required.

Component	PCR target		
Component	Roundup Ready™ Soybean	Lectin	
RR Soybean Master Mix (green disc)	7.5 µL	_	
Soya Master Mix (purple disc)	_	7.5 µL	
General Master Mix (white disc)	12.5 μL	12.5 µL	

- 2. Mix thoroughly by vortexing, then distribute 20 μL to the appropriate reaction wells or tubes. See Figure 2 for an example plate layout.
- 3. Add 5 µL of each sample DNA (10–25 ng/µL), mock-purified sample (negative extraction control), or nuclease-free water (no-template control) to the appropriate wells for both Roundup Ready™ Soybean and lectin PCRs.
- 4. Add 5 μL of each Roundup Ready™ Soya Standard dilution to the appropriate wells for both Roundup Ready™ Soybean and lectin PCRs.
- 5. Seal each plate or tube, mix, then centrifuge briefly to bring the contents to the bottom.

		1	2		3	4	
	1	2	3	4	5	6	7
Α							
В		S 2E5	U 1		S 2E5	U 1	
С		S 2E4	U 1		S 2E4	U 1	
D		S 2E3	U 2		S 2E3	U 2	
Е		S 200	U 2		S 200	U 2	
F		S 20			S 20		
G		Neg.Ext.			Neg. Ext.		
Н		N NTC			N NTC		

Figure 2 Example plate layout

In this example, each PCR series includes two unknown samples (U) in duplicate and the recommended dilutions of Roundup Ready™ Soya Standard (S). Negative extraction control (Neg.Ext.; N) and no-template control reactions (NTC; N) are also included.

- ① Standard dilutions and negative controls, Roundup Ready™ Soybean PCR
- ② Unknown samples, Roundup Ready™ Soybean PCR
- (3) Standard dilutions and negative controls, lectin PCR
- (4) Unknown samples, lectin PCR

Set up and run the real-time PCR instrument

See the appropriate instrument user guide for detailed instructions to set up and run the real-time PCR instrument.

Note: The real-time PCR instrument must be calibrated with the following dye before use: FAM™.

- 1. Set up the real-time PCR instrument using the following settings:
 - Reaction volume: 25 μL
 - Passive reference dye: ROX™ dye included
 - TaqMan™ probe reporter: FAM™ dye
 - TaqMan™ probe quencher: MGB

• Thermal cycler settings:

Setting	Stage 1 Enzyme activation	Stage 2 PCR		
Number of cycles	1 (Hold)	50		
Number of cycles	i (Hold)	Denature	Anneal/extend ^[1]	
Temperature	95°C	95°C	60°C	
Time	10 minutes	15 seconds	1 minute	

^[1] For each PCR cycle, the fluorescence acquisition takes place during the annealing/extension stage.

2. Load the reactions, run the thermal cycler program and collect real-time amplification data.

Analyze results

The general process for analyzing results is described in this section. The details of data analysis depend on the real-time PCR instrument that you use. See the appropriate user guide for instructions on how to analyze your data.

- 1. View the amplification plots for all reactions to make sure that they appear normal. See Figure 3 and Figure 4 for example data.
- 2. Use the **Auto** instrument setting to set the baseline.
- 3. Set the FAM™ threshold to 0.1.
- 4. Confirm that results for the negative controls and Roundup Ready™ Soya Standard are as expected. For unexpected control results, see Appendix A, "Troubleshooting".

Sample type	Roundup Ready™ soybean PCR series	Lectin PCR series			
Negative extraction control or no-template control	No amplification ^[1]	No amplification [1]			
Roundup Ready™ Soya Standard dilution	Amplification should be detected in all five dilutions for both Roundup Ready™ soybean and lectin PCR series (see Figure 3).				
series	The standard curves derived from the dilution series should meet the following requirements (see Figure 4):				
	 The efficiency of the curve should be 86–110% (slope between –3.7 and –3.1). 				
	• The correlation coefficient (R ²) should be >0.98.				

^[1] Amplification in a negative control indicates contamination. The assay should be repeated.

Chapter 2 Methods Analyze results

5. Determine whether the Roundup Ready™ soybean and lectin targets can be detected and quantified for each unknown sample.

Table 2 Quantifiability of samples

Unknown sample result	Interpretation
No amplification detected.	Not detected.
Amplification detected and the sample C_t falls within the corresponding standard curve.	Detected and quantifiable.
Amplification detected and the sample C_t is greater than the highest C_t of the corresponding standard curve.	Detected but not quantifiable. The concentration of DNA in the sample is too low.
Amplification detected and the sample C_t is lower than the lowest C_t of the corresponding standard curve.	Detected but not quantifiable. The concentration of DNA in the sample is too high. See "Input DNA requirements" on page 8.

Table 3 Interpretation of results

Lectin PCR	Roundup Ready™ Soybean PCR	Interpretation	
Quantifiable	Not detected	No Roundup Ready™ soybean detected.	
Quantifiable	Not quantifiable	The amount of Roundup Ready™ soybean detected is lower than the limit of quantification.	
Quantifiable	Quantifiable	The number of copies of Roundup Ready™ soybean with respect to the number of copies of lectin can be calculated (step 6).	
Not quantifiable	Not detected	No Roundup Ready™ soybean detected and the amount of soy is lower than the limit of quantification.	
Not quantifiable	Not quantifiable	The amounts of soy and Roundup Ready™ soybean detected are lower than the limit of quantification.	
Not detected	Not detected	No soy or Roundup Ready™ soybean detected, or inhibitors are present. See "Test for the presence of inhibitors in the sample" on page 14.	

- 6. If the results for both PCR series for the unknown samples are quantifiable, calculate the percentage of Roundup Ready™ soybean as follows:
 - a. Calculate the relative quantity of Roundup Ready™ soybean and lectin with respect to the Roundup Ready™ Soya Standard in each PCR (Figure 4).
 - b. Calculate the percentage of Roundup Ready™ soybean:

Your instrument software may be set up to automatically perform this calculation.

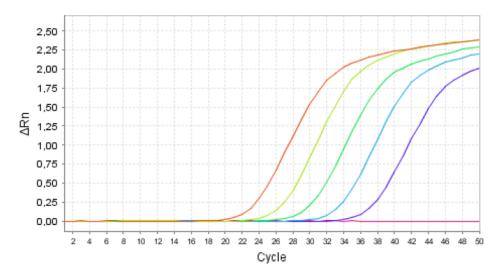


Figure 3 Example amplification curves for Roundup Ready™ Soya Standard dilution series.

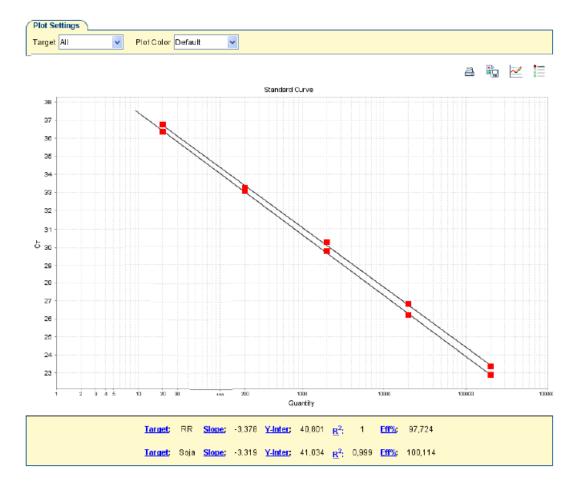


Figure 4 Example standard curves for total soya and Roundup Ready™ soybean using Roundup Ready™ Soya Standard dilution series.

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Test for the presence of inhibitors in the sample

In this experiment, PCR amplification of the Roundup Ready™ Soya Standard in the presence and absence of a sample that is suspected of containing PCR inhibitors is compared. Set up and run two PCRs:

Component	Positive control	Test
Soya Master Mix (purple disc)	7.5 µL	7.5 µL
General Master Mix (white disc)	12.5 μL	12.5 µL
Roundup Ready™ Soya Standard, 1:10 dilution (4000 copies/µL)	1 µL	1 μL
Nuclease-free water	5 μL	_
Test sample	_	5 μL

The sample does not contain inhibitors if the amplification of both PCR reactions is similar.



Troubleshooting

Observation	Possible cause	Recommended action	
In the Positive Control wells, no target-specific and no IPC signals are detected.	PCR amplification failed.	Check that the thermal cycler settings and amplification program are correct.	
In the negative extraction control wells, target-specific and IPC signals are detected.	Contamination occurred during the DNA extraction procedure.	 Contamination may be due to errors in sample handling, reagent contamination, or environmental contamination. Check that the DNA extraction protocol was performed correctly. Take care to avoid contamination during sample homogenization: decontaminate grinding equipment or homogenizer with 10% bleach or DNAZap™ Solutions (Cat. No. AM9890). Decontaminate benchtop surfaces and other equipment where the DNA extraction process is performed with 10% bleach or DNAZap™ Solutions. If necessary, use fresh reagents and repeat the DNA extraction. 	
In the no-template control wells, target-specific and IPC signals are detected.	Contamination occurred during PCR.	 Contamination may be due to errors in sample handling, reagent contamination, or environmental contamination. Decontaminate benchtop surfaces and other equipment where PCR is performed with 10% bleach or DNAZap™ Solutions (Cat. No. AM9890). Use fresh reagents and repeat the PCR. Set up the Positive Control PCR reactions last to avoid cross-contamination. 	
In unknown wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA existed in the samples, resulting in preferential amplification of the target-specific DNA.		
In unknown wells, no IPC or target-specific signal is detected.	Excess sample DNA was used in PCR; the recommended maximum is 250 ng. PCR inhibitors were present in the sample DNA.	Repeat the PCR with the correct amount of DNA. If DNA quantification is not possible, dilute the DNA sample. Repeat the DNA extraction. If the problem persists, contact Technical Support.	



Supplemental information

UNE-EN ISO 9001 certification

Health in Code S.L. is certified against the standard UNE-EN ISO 9001:2015 "Quality management systems" for the design, development, manufacture, and commercialization of kits for genetic analysis.

UNE-EN ISO 14001 certification

Health in Code S.L. is certified against the standard UNE-EN ISO 14001:2015 "Environmental Management Systems" for the design, development, manufacture, and commercialization of kits for genetic analysis.



Good laboratory practices for PCR

Note: Spin tubes/plates before performing PCR. Spinning of PCR tubes is most easily accomplished by using a centrifuge designed for PCR tubes or plates. Follow manufacturer instructions for loading tubes/plates.

To avoid amplicon contamination of samples, follow these guidelines when preparing or handling samples for PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified products or used during sample preparation).
- Change gloves whenever 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.
- Do not open reaction tubes after PCR.
- Do not autoclave reaction tubes after PCR.
- Clean lab benches and equipment periodically with 10% bleach solution or DNAZap™ Solutions (Cat. No. AM9890) according to the Thermo Fisher Scientific PCR Decontamination Protocol. After cleaning with bleach we recommend a rinse with distilled water or an ethanol solution because bleach will rust stainless steel. Note that minor discoloration of metal parts may occur.

For additional information, refer to EN ISO 22174:2005 or www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html.

Plate layout suggestions

- Separate different targets by a row if enough space is available.
- Put at least one well between unknown samples and controls if possible.
- Separate negative and positive controls by one well if possible.
- Place replicates of one sample for the same target next to each other.
- Start with the unknown samples and put controls at the end of the row or column.
- Put positive controls in one of the outer rows or columns if possible.
- Consider that caps for PCR tubes come in strips of 8 or 12.

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, visit thermofisher.com/support.

Chemical safety



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

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



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



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Appendix D Safety Biological hazard safety

Biological hazard safety



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



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

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



Documentation and support

Food safety support

Website: https://www.thermofisher.com/us/en/home/industrial/food-beverage/food-microbiology-testing.html

Health in Code website for Certificates of Analysis and other product documentation: https://portal.imegen.es/en/certificate-of-analysis/

Support email:

- Europe, Middle East, Africa: microbiology.techsupport.uk@thermofisher.com
- North America: microbiology@thermofisher.com

Phone: Visit **thermofisher.com/support**, select the link for phone support, then select the appropriate country from the dropdown list.

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.

Related documentation

Document	Publication number
KingFisher™ Flex Purification System User Guide	MAN0019870
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4378657
Applied Biosystems™ 7500/7500 Fast Real-Time PCR System: Maintenance Guide	4387777

Limited product warranty

Life Technologies Corporation and its affiliates 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 questions, contact Life Technologies at www.thermofisher.com/support.

