

Custom Reverse Transcription Pools and Custom Preamplification Pools with TaqMan[®] MicroRNA Assays

USER GUIDE

Publication Number 4465407

Revision E



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

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. 4465407

Revision	Date	Description
E	13 September 2019	<ul style="list-style-type: none">• Corrected thermal protocol for reverse transcription.• Corrected the amount of time that the RT reaction can be stored.• Added QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems.• Updated thermal cyclers.• Removed duplicated step to centrifuge the tubes and the plates in the instructions to dilute the preamplification reaction.• Removed 4°C hold step after real-time PCR.• Updated the guidelines for data analysis.
D	4 February 2019	<ul style="list-style-type: none">• Updated reverse transcription thermal cycling conditions.• Added new instruments, Master Mixes, and other products applicable to the workflows.• Updated data analysis information.• Rebranded and streamlined the protocol.
C	January 2013	Baseline for this revision history.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Trademarks: All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a registered trademark of Roche Molecular Systems, Inc., used under permission and license. AmpErase is a trademark of Roche Molecular Systems, Inc.

©2019 Thermo Fisher Scientific Inc. All rights reserved.

Contents

■	CHAPTER 1	Product information	5
		Description	5
		Required materials not supplied	5
		Workflow	7
■	CHAPTER 2	TaqMan[®] MicroRNA Assays with custom RT pools and custom preamplification pools	8
		Prepare custom pools	8
		Prepare the custom RT primer pool	8
		Prepare the custom preamplification primer pool	9
		Perform reverse transcription	9
		Perform the preamplification	11
		Dilute the preamplification reaction	12
		Perform PCR amplification	12
		Prepare the PCR Reaction Mix	12
		Set up and run the real-time PCR instrument	13
■	CHAPTER 3	TaqMan[®] MicroRNA Assays with custom RT pools and no preamplification	14
		Prepare the custom RT primer pool	14
		Perform reverse transcription	15
		Perform PCR amplification	16
		Prepare the PCR Reaction Mix	16
		Set up and run the real-time PCR instrument	17
■	CHAPTER 4	Analyze data	18
		Perform data analysis	18
■	APPENDIX A	Safety	19
		Chemical safety	20
		Biological hazard safety	21

■ APPENDIX B	Documentation and support	22
	Related documentation	22
	Customer and technical support	23
	Limited product warranty	24



Product information

- Description 5
- Required materials not supplied 5
- Workflow 7

Description

This user guide describes how to pool up to 96 individual reverse transcriptase (RT) primers and TaqMan® MicroRNA Assays for preamplification. The standard microRNA protocol calls for an individual RT reaction for each target miRNA. This guide explains a multiplexed RT step for pools composed of up to 96 individual RT primers or microRNA assays for preamplification. These pools can be used with the matching TaqMan® MicroRNA Assays on plates prepared by the user, or through our custom plating service. For details, contact Specialty_Cards@lifetech.com and Specialty_Plates@lifetech.com.

Due to the complexity of the pool, some assays may exhibit less than optimal performance. We recommend that you include a no-template control (NTC) and verify pool performance with the individual RT reaction.

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 1 Reagent for reverse transcription

Item	Source
TaqMan® MicroRNA Reverse Transcription Kit ^[1]	4366597

^[1] Contains 10X RT buffer, dNTP mix w/dTTP (100 mM), RNase Inhibitor (20U/μL), and MultiScribe™ Reverse Transcriptase enzyme (50U/μL).

IMPORTANT! Do not use the TaqMan® Gene Expression Master Mix contained in the TaqMan® PreAmp Master Mix Kit.

Table 2 Master Mixes and TaqMan® Assays

Item	Source
Assays	
TaqMan® MicroRNA Assays	thermofisher.com/taqmanmirna
Preamplification Master Mix	
TaqMan® PreAmp Master Mix (2X)	4488593
PCR Master Mixes	
TaqMan® Universal Master Mix II, no UNG (2X)	4440041
TaqMan® Universal PCR Master Mix, no AmpErase™ UNG (2X)	4324018
TaqMan® Fast Advanced Master Mix	4444963

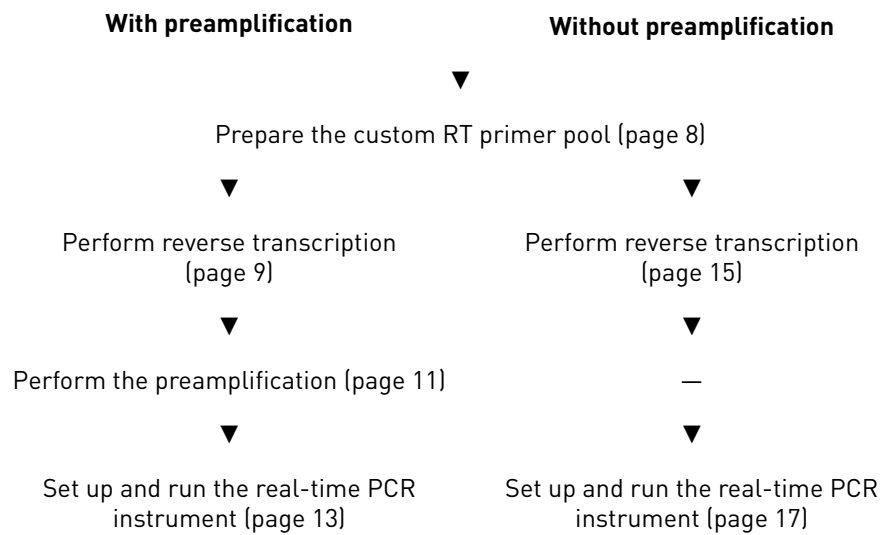
Table 3 Real-time PCR instruments and thermal cyclers

Item	Source
Real-time PCR instruments	
QuantStudio™ 6 Pro Real-Time PCR System or QuantStudio™ 7 Pro Real-Time PCR System	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	
ViiA™ 7 Real-Time PCR System	
StepOnePlus™ Real-Time PCR System	
7900HT Real-Time PCR System or 7900HT Fast Real-Time PCR System	
7500 Real-Time PCR System or 7500 Fast Real-Time PCR System	
Thermal cyclers	
Veriti™ Thermal Cycler	Contact your local sales office.
SimpliAmp™ Thermal Cycler	
MiniAmp™ Thermal Cycler	
ProFlex™ PCR System	

Table 4 Other consumables

Item	Source
Plastics consumables	thermofisher.com/plastics
Nuclease-free Water	AM9937
1× TE Buffer	12090015

Workflow



2

TaqMan[®] MicroRNA Assays with custom RT pools and custom preamplification pools

- Prepare custom pools 8
- Perform reverse transcription 9
- Perform the preamplification 11
- Dilute the preamplification reaction 12
- Perform PCR amplification 12

Note: Preamplification is required if total RNA is 1–350 ng. It is recommended to detect low-expressing miRNAs.

Prepare custom pools

Prepare the custom RT primer pool

Each TaqMan[®] MicroRNA Assay contains one 5× RT primer. Up to 96 primers can be pooled into one RT reaction.

When creating a pool, we recommend running a no-template control (NTC) reaction for each assay in the pool to check the background. An NTC reaction uses nuclease-free water in place of sample in the RT reaction.

1. In a 1.5-mL microcentrifuge tube, combine 10 µL of each 5× RT primer.
2. Add 1× TE buffer to bring the final volume to 1000 µL.

Number of assays	Total pooled volume	1× TE volume	RT primer pool total volume
12	120 µL	880 µL	1000 µL
16	160 µL	840 µL	1000 µL
24	240 µL	760 µL	1000 µL
32	320 µL	680 µL	1000 µL
48	480 µL	520 µL	1000 µL
64	640 µL	360 µL	1000 µL
96	960 µL	40 µL	1000 µL

The final concentration of each of the RT primers in the RT primer pool is 0.05×.

This volume is sufficient for 148 × 15-μL RT reactions with 12% overage allotted for pipetting loss.

The RT primer pool can be stored at -20°C for up to two months.

Prepare the custom preamplification primer pool

The assays contain a 20× mix of miRNA-specific forward and reverse primers, and miRNA-specific probe. Prepare the preamplification pool so that the final concentration of each assay is 0.2×.

1. In a 1.5 mL microcentrifuge tube, combine 10 μL of each 20× assay.
2. Add 1× TE buffer to bring the final volume to 1000 μL.

Number of assays	Total pooled volume	1× TE volume	Preamplification primer pool total volume
12	120 μL	880 μL	1000 μL
16	160 μL	840 μL	1000 μL
24	240 μL	760 μL	1000 μL
32	320 μL	680 μL	1000 μL
48	480 μL	520 μL	1000 μL
64	640 μL	360 μL	1000 μL
96	960 μL	40 μL	1000 μL

The final concentration of each of the preamplification primers in the preamplification primer pool is 0.2×.

This volume is sufficient for 235 × 25-μL preamplification reactions.

The preamplification primer pool can be stored at -20°C for up to 2 months.

Perform reverse transcription

All of the reagents needed for the reverse transcription reaction are included in the TaqMan® MicroRNA Reverse Transcription Kit except for the RT primer pool.

1. Prepare the RT Reaction Mix on ice in an appropriately-sized microcentrifuge tube according to the following table.

Note: Do not vortex the MultiScribe™ Reverse Transcriptase or the RT Reaction Mix.

The RT primer pool was prepared in “Prepare the custom RT primer pool” on page 8.

Component	Volume (1 sample) ^[1]	Volume (3 samples) ^[2,3]
RT primer pool	6.00 µL	20.25 µL
dNTPs with dTTP (100 mM)	0.30 µL	1.01 µL
MultiScribe™ Reverse Transcriptase (50 U/µL)	3.00 µL	10.13 µL
10X RT buffer	1.50 µL	5.06 µL
RNase Inhibitor (20 U/µL)	0.19 µL	0.64 µL
Nuclease-free Water	1.01 µL	3.41 µL
Total RT Reaction Mix volume	12.00 µL	40.5 µL

^[1] Sufficient for up to 96 assays in the PCR step with no replicates.

^[2] Includes 12.5% overage for pipetting loss.

^[3] Sufficient for up to 96 assays in the PCR step with 4 replicates of each assay.

- Invert to mix, then centrifuge briefly to collect the contents at the bottom of the tube.
- Add 12 µL of RT Reaction Mix to each well of a 96-well plate or to reaction tubes.
- Add 3 µL of total RNA (or nuclease-free water for no-template control) to each well of the reaction plate or to the reaction tubes.
3 µL of the RNA sample should contain 1–350 ng of total RNA.
- Seal the reaction plate or tubes, invert to mix, then centrifuge briefly to collect the contents at the bottom.
- Incubate on ice for 5 minutes.
- Place the reaction plate or tubes into a thermal cycler, then incubate using a standard ramp speed and a reaction volume of 15 µL.

Step	Time	Temperature
1	30 minutes	16°C
2	30 minutes	42°C
3	5 minutes	85°C
4	Hold until stopped	4°C

Proceed to the preamplification reaction or store the RT reaction product at –25°C to –15°C for up to one week.

Perform the preamplification

1. In an appropriately-sized microcentrifuge tube, prepare the Preamplification Reaction Mix according to the following table.

Component	Volume (1 sample) ^[1]
RT reaction product ^[2]	2.5 µL
TaqMan® PreAmp Master Mix (2X)	12.50 µL
Preamplification primer pool ^[3]	3.75 µL
Nuclease-free Water	6.25 µL
Total Preamplification Reaction Mix volume	25.00 µL

^[1] Add 12.5% overage.

^[2] From "Perform reverse transcription" on page 9.

^[3] From "Perform the preamplification" on page 11.

2. Invert to mix thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.
3. Add 25 µL of the Preamplification Reaction Mix to each well of a 96-well plate or to reaction tubes.
4. Seal the reaction plate or tubes, invert to mix, then centrifuge briefly to collect the contents at the bottom.
5. Place the reaction plate or tubes into a thermal cycler, then incubate using a standard ramp speed and the following settings.

Step	Temperature	Time	Cycles
Enzyme activation	95°C	10 minutes	1
Anneal	55°C	2 minutes	1
Extend	72°C	2 minutes	1
Denature	95°C	15 seconds	12
Anneal / Extend	60°C	4 minutes	
Enzyme Inactivation	99.9°C	10 minutes	1
Hold	4°C	Hold	Hold

Proceed to the next section.

Dilute the preamplification reaction

1. Remove the reaction plate or tubes from the thermal cycler, then briefly centrifuge to collect the contents at the bottom of the wells or the tubes.
2. Add 175 μL of 0.1 \times TE, pH 8.0 to each well or tube.
The final volume is 200 μL .
3. Seal the plate or tubes, invert to mix, then centrifuge briefly to collect the contents at the bottom.

Place the diluted preamplification reaction product on ice and proceed immediately to the real-time PCR reactions in the next section, or store at -25°C to -15°C for up to one week.

Perform PCR amplification

Prepare the PCR Reaction Mix

1. Mix the PCR Master Mix thoroughly but gently.
2. Prepare the PCR Reaction Mix in an appropriately-sized microcentrifuge tube, according to the following table.

Component	Volume for 1 reaction	
	384-well (0.1-mL) or 96-well fast (0.1-mL) plates	96-well standard (0.2-mL) plate
TaqMan® MicroRNA Assays (20 \times)	0.50 μL	1.0 μL
Diluted preamplification product ^[1]	0.10 μL	0.20 μL
PCR Master Mix ^[2,3]	5.00 μL	10.00 μL
Nuclease-free Water	4.40 μL	8.80 μL
Total PCR Reaction Mix volume	10.00 μL	20.00 μL

^[1] From "Dilute the preamplification reaction" on page 12.

^[2] See Table 2 on page 6.

^[3] Versions of these Master Mixes with or without UNG may be used. The step for UNG incubation is not recommended.

3. Vortex to mix the PCR Reaction Mix thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.
4. Transfer the appropriate volume of PCR Reaction Mix to each well of an optical reaction plate.
5. Seal the plate with MicroAmp™ Optical Adhesive Film, then vortex briefly to mix the contents.
6. Centrifuge the plate briefly to collect the contents at 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.

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

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

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

2. Set the thermal cycling conditions as shown in the tables below.

Note: The UNG incubation step is not recommended, even if a Master Mix containing UNG is used.

Table 5 TaqMan® Universal Master Mix II, no UNG or TaqMan® Universal PCR Master Mix, no AmpErase™ UNG (any compatible instrument, standard cycling mode)

Step	Temperature	Time	Cycles
Enzyme activation	95°C	10 minutes	1
Denature	95°C	15 seconds	40
Anneal / Extend	60°C	60 seconds	

Table 6 TaqMan® Fast Advanced Master Mix (with QuantStudio™ systems, ViiA™ 7 Real-Time PCR System, StepOnePlus™ Real-Time PCR System, or 7900HT Real-Time PCR System/7900HT Fast Real-Time PCR System, fast cycling mode)

Step	Temperature	Time	Cycles
Enzyme activation	95°C	20 seconds	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

Table 7 TaqMan® Fast Advanced Master Mix (with 7500 Real-Time PCR System/7500 Fast Real-Time PCR System, fast cycling mode)

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

3. Start the run.

3

TaqMan[®] MicroRNA Assays with custom RT pools and no preamplification

- Prepare the custom RT primer pool 14
- Perform reverse transcription 15
- Perform PCR amplification 16

Preamplification is not required if total RNA is 350–1000 ng.

Prepare the custom RT primer pool

Each TaqMan[®] MicroRNA Assay contains one 5× RT primer. Up to 96 primers can be pooled into one RT reaction.

When creating a pool, we recommend running a no-template control (NTC) reaction for each assay in the pool to check the background. An NTC reaction uses nuclease-free water in place of sample in the RT reaction.

1. In a 1.5-mL microcentrifuge tube, combine 10 µL of each 5× RT primer.
2. Add 1× TE buffer to bring the final volume to 1000 µL.

Number of assays	Total pooled volume	1× TE volume	RT primer pool total volume
12	120 µL	880 µL	1000 µL
16	160 µL	840 µL	1000 µL
24	240 µL	760 µL	1000 µL
32	320 µL	680 µL	1000 µL
48	480 µL	520 µL	1000 µL
64	640 µL	360 µL	1000 µL
96	960 µL	40 µL	1000 µL

The final concentration of each of the RT primers in the RT primer pool is 0.05×. This volume is sufficient for 148 × 15-µL RT reactions with 12% overage allotted for pipetting loss.

The RT primer pool can be stored at –20°C for up to two months.

Perform reverse transcription

All of the reagents needed for the reverse transcription reaction are included in the TaqMan® MicroRNA Reverse Transcription Kit except for the RT primer pool.

1. Prepare the RT Reaction Mix on ice in an appropriately-sized microcentrifuge tube according to the following table.

Note: Do not vortex the MultiScribe™ Reverse Transcriptase or the RT Reaction Mix.

The RT primer pool was prepared in “Prepare the custom RT primer pool” on page 8.

Component	Volume (1 sample) ^[1]	Volume (3 samples) ^[2,3]
RT primer pool	6.00 µL	20.25 µL
dNTPs with dTTP (100 mM)	0.30 µL	1.01 µL
MultiScribe™ Reverse Transcriptase (50 U/µL)	3.00 µL	10.13 µL
10X RT buffer	1.50 µL	5.06 µL
RNase Inhibitor (20 U/µL)	0.19 µL	0.64 µL
Nuclease-free Water	1.01 µL	3.41 µL
Total RT Reaction Mix volume	12.00 µL	40.5 µL

^[1] Sufficient for up to 96 assays in the PCR step with no replicates.

^[2] Includes 12.5% overage for pipetting loss.

^[3] Sufficient for up to 96 assays in the PCR step with 4 replicates of each assay.

2. Invert to mix, then centrifuge briefly to collect the contents at the bottom of the tube.
3. Add 12 µL of RT Reaction Mix to each well of a 96-well plate or to reaction tubes.
4. Add 3 µL of total RNA (or nuclease-free water for no-template control) to each well of the reaction plate or to the reaction tubes.
 3 µL of the RNA sample should contain 350–1000 ng of total RNA.
5. Seal the reaction plate or tubes, invert to mix, then centrifuge briefly to collect the contents at the bottom.
6. Incubate on ice for 5 minutes.

- Place the reaction plate or tubes into a thermal cycler, then incubate using a standard ramp speed and a reaction volume of 15 µL.

Step	Time	Temperature
1	30 minutes	16°C
2	30 minutes	42°C
3	5 minutes	85°C
4	Hold until stopped	4°C

The RT reaction product can be stored at –25°C to –15°C for up to one week.

Perform PCR amplification

Prepare the PCR Reaction Mix

- Mix the PCR Master Mix thoroughly but gently.
- Prepare the PCR reaction mix in an appropriately-sized microcentrifuge tube, according to the following table.

Component	Volume for 1 reaction	
	384-well (0.1-mL) or 96-well fast (0.1-mL) plates	96-well standard (0.2-mL) plate
TaqMan® MicroRNA Assays (20×)	0.50 µL	1.0 µL
RT product ^[1]	0.08 µL	0.16 µL
PCR Master Mix ^[2,3]	5.00 µL	10.00 µL
Nuclease-free Water	4.42 µL	8.84 µL
Total PCR Reaction Mix volume	10.00 µL	20.00 µL

^[1] From “Perform reverse transcription” on page 15.

^[2] See Table 2 on page 6.

^[3] Versions of these Master Mixes with or without UNG may be used. The step for UNG incubation is not recommended.

- Vortex to mix the PCR Reaction Mix thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.
- Transfer the appropriate volume of PCR Reaction Mix to each well of an optical reaction plate.
- Seal the plate with MicroAmp™ Optical Adhesive Film, then vortex briefly to mix the contents.
- Centrifuge the plate briefly to collect the contents at 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.

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

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

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

2. Set the thermal cycling conditions as shown in the tables below.

Note: The UNG incubation step is not recommended, even if a Master Mix containing UNG is used.

Table 8 TaqMan® Universal Master Mix II, no UNG or TaqMan® Universal PCR Master Mix, no AmpErase™ UNG (any compatible instrument, standard cycling mode)

Step	Temperature	Time	Cycles
Enzyme activation	95°C	10 minutes	1
Denature	95°C	15 seconds	40
Anneal / Extend	60°C	60 seconds	

Table 9 TaqMan® Fast Advanced Master Mix (with QuantStudio™ systems, ViiA™ 7 Real-Time PCR System, StepOnePlus™ Real-Time PCR System, or 7900HT Real-Time PCR System/7900HT Fast Real-Time PCR System, fast cycling mode)

Step	Temperature	Time	Cycles
Enzyme activation	95°C	20 seconds	1
Denature	95°C	1 second	40
Anneal / Extend	60°C	20 seconds	

Table 10 TaqMan® Fast Advanced Master Mix (with 7500 Real-Time PCR System/7500 Fast Real-Time PCR System, fast cycling mode)

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

3. Start the run.



Analyze data

Perform data analysis

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

An automatic baseline and a threshold value of 0.2 are recommended as a starting point.

Perform the following actions, if necessary:

- 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 each replicate group.

Perform additional analysis using the following software tools. This software is free and can be downloaded from the Thermo Fisher Scientific web site.

Software	Resource
Relative Quantification application	thermofisher.com/cloud
Standard Curve application	
ExpressionSuite™ Software	thermofisher.com/expressionsuite
DataAssist™ Software	thermofisher.com/dataassist



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.



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

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

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

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.
<i>Introduction to Gene Expression Getting Started Guide</i>	4454239
<i>Understanding Your Shipment</i>	MAN0017153
<i>Custom TaqMan[®] Assays Design and Ordering Guide</i>	4367671
<i>TaqMan[®] PreAmp Master Mix User Guide</i>	4384557
<i>TaqMan[®] PreAmp Master Mix Quick Reference</i>	4384556
QuantStudio[™] 6 Pro and 7 Pro Real-Time PCR Systems	
<i>QuantStudio[™] 6 Pro and 7 Pro Real-Time PCR Systems User Guide</i>	MAN0018045
QuantStudio[™] 3 or 5 Real-Time PCR System	
<i>QuantStudio[™] 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide</i>	MAN0010407
<i>QuantStudio[™] Design and Analysis Desktop Software User Guide</i>	MAN0010408
QuantStudio[™] 6 / QuantStudio[™] 7 Flex Real-Time PCR System	
<i>QuantStudio[™] 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide</i>	4489821
<i>QuantStudio[™] 6 and 7 Flex Real-Time PCR System Software Getting Started Guide</i>	4489822
QuantStudio[™] 12K Flex Real-Time PCR System	
<i>QuantStudio[™] 12K Flex Real-Time PCR System Maintenance and Administration Guide</i>	4470689
<i>QuantStudio[™] 12K Flex Real-Time PCR System: Multi-Well Plates and Array Card Experiments User Guide</i>	4470050
StepOne[™] or StepOnePlus[™] Real-Time PCR System	
<i>StepOne[™] and StepOnePlus[™] Real-Time PCR Systems Installation, Networking and Maintenance User Guide</i>	4376782
<i>Applied Biosystems[™] StepOne[™] and StepOnePlus[™] Real-Time PCR Systems Relative Standard Curve and Comparative C_t Experiments Getting Started Guide</i>	4376785
ViiA[™] 7 Real-Time PCR System	
<i>Applied Biosystems[™] ViiA[™] 7 Real-Time PCR System User Guide: Calibration, Maintenance, Networking, and Security</i>	4442661
<i>Applied Biosystems[™] ViiA[™] 7 Real-Time PCR System Getting Started Guide</i>	4441434

Document	Pub. No.
7500/7500 Fast Real-Time PCR System	
<i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide</i>	4347828
<i>Applied Biosystems™ 7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Standard Curve and Comparative C_t Experiments</i>	4387783
Data analysis	
<i>Real-Time PCR Systems Chemistry Guide: Applied Biosystems™ 7900HT Fast Real-Time PCR System and 7300/7500 Real-Time PCR Systems</i>	4348358
<i>Applied Biosystems™ 7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative C_T Getting Started Guide</i>	4364016
<i>Applied Biosystems™ 7900HT Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide</i>	4364014
<i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve</i>	4347825
<i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Quantitation using Comparative C_t</i>	4347824
<i>Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C_t Experiments Getting Started Guide</i>	4376785
<i>Applied Biosystems™ Relative Quantitation Analysis Module User Guide</i>	MAN0014820
<i>Applied Biosystems™ Standard Curve Analysis Module User Guide</i>	MAN0014819

Customer and technical support

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

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

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



Limited product warranty

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

