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TaqMan™ RNA-to-C_T™ 1-Step Kit

USER GUIDE

for use with:
TaqMan™ Gene Expression Assays
Custom TaqMan™ Gene Expression Assays

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E	14 April 2023	• The storage conditions for the 2X TaqMan™ RT-PCR Mix and the 40X TaqMan™ RT Enzyme Mix were updated. • The product description was updated. • The real-time PCR instrument list was updated. • The required materials list was updated. • The experiment protocol was streamlined. • Branding and trademarks were updated.
D	4 May 2012	Baseline for this revision history.

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

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Product information

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About the TaqMan™ RNA-to-C_T™ 1-Step Kit

The Applied Biosystems™ TaqMan™ RNA-to-C_T™ 1-Step Kit provides TaqMan™ reagents necessary to perform one-step RT–PCR quantitation experiments on a real-time PCR system.

The kit is designed to work with TaqMan™ Gene Expression Assays and Custom TaqMan™ Gene Expression Assays. For information on gene expression assays, see *TaqMan™ Gene Expression Assays User Guide—single-tube assays* (Pub. No. 4333458).

Contents and storage

Contents	Cat. No. 4392653 (40 × 50 µL reactions)	Cat. No. 4392938 (200 × 50 µL reactions)	Cat. No. 4392656 (2,000 × 50 µL reactions)	Storage[1]
2X TaqMan™ RT-PCR Mix	1 mL	5 mL	10 × 5 mL	–25°C to –15°C on receipt, protect from light 2–8°C after first use, protect from light
40X TaqMan™ RT Enzyme Mix	50 µL	250 µL	10 × 250 µL	–25°C to –15°C

[1] See packaging for expiration date.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Table 1 Real-time PCR instrument

Item	Source
StepOne™ Real-Time PCR System	Contact your local sales office
StepOnePlus™ Real-Time PCR System	
QuantStudio™ 3 or 5 Real-Time PCR System	
QuantStudio™ 6 or 7 Flex Real-Time PCR System	
QuantStudio™ 6 Pro or 7 Pro Real-Time PCR System	
QuantStudio™ 12K Flex Real–Time PCR System	
7500/7500 Fast Real-Time PCR Systems	
7900HT Real-Time PCR System	

Table 2 Other materials and equipment required for the workflow

Item	Source
Software	
Primer Express™ Software	4363991
(Optional) Relative Quantification application	Available on the Thermo Fisher™ Connect Platform
(Optional) Standard Curve application	
Equipment	
Centrifuge, with adapter for 96-well plates or 384-well plates	MLS
Microcentrifuge	MLS
Vortex mixer	MLS
Pipettes	MLS
Tubes, plates, and other consumables	
Tubes, plates, and film	**thermofisher.com/plastics**
Aerosol-resistant barrier pipette tips	MLS
Disposable gloves	MLS

Item	Source
Reagents	
Nuclease-free water	MLS
TaqMan™ DNA Template Reagents	401970
TaqMan™ RNase P Detection Reagents Kit	4316831
(Optional) TE, pH 8.0, RNase-free	AM9849

Workflow

Prepare, run, and analyze an experiment

Design the experiment (page 8)

Prepare the RT-PCR reactions (page 9)

Set up and run the RT-PCR Reaction Mix plate (page 12)

Analyze the results (page 13)

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Design the experiment

For detailed information on designing and setting up a quantification experiment, see the user guide for your real-time PCR system.

1. Select the type of assay to use.

   Note: The kit is designed to work with TaqMan™ Gene Expression Assays and Custom TaqMan™ Gene Expression Assays. For detailed information on designing custom gene expression assays, see "Guidelines for custom–designed assays" on page 14.

   Note: The reverse transcriptase enzyme contained in this kit is produced using an *E. coli* expression vector containing a proprietary version of the MMLV *pol* gene (GenBank™ accession no. J02255) expressed from pET-24(+). It is possible that a minimal amount of the expression vector could be carried over into the final master mix formulation. To target MMLV, a related virus, or any of the plasmid sequence, we recommend designing primer sequences not contained in the expression vector.

2. Select a method to determine the quantity of target in the samples from the following list of analyses:
   - **Standard curve—**Uses a standard curve to determine the absolute quantity of a target in a sample. This analysis is typically used to quantify viral load.
   - **Relative standard curve—**Uses standard curves to determine the change in expression of a target in a sample relative to the same target in a reference sample. This analysis is used for relative quantification if the amplification efficiency of the target and the amplification efficiency of the endogenous control are not approximately equal.
   - **Comparative C_t—**Uses arithmetic formulas to determine the change in expression of a target in a sample relative to the same target in a reference sample. This analysis is best for high–throughput measurements of relative gene expression of many genes in many samples.

3. Determine the numbers of samples in your experiment according to the following categories:
   - **Samples or Unknowns—**Samples in which the quantity of the target is unknown.
   - **Standards** (standard curve and relative standard curve experiments)—Samples that contain known standard quantities. A set of standards containing a range of known quantities is a standard dilution series.
   - **Reference sample** (relative standard curve and comparative C_t experiments)—The sample used as the basis for relative quantification results.

Note: We recommend performing at least 3 technical replicates of each reaction.

Prepare the RT-PCR reactions

About the RNA template

With the TaqMan™ RNA-to-C_T™ 1-Step Kit, the target template is the RNA sequence that you want to amplify and detect.

RNA template quality

For optimal performance, use purified total RNA or mRNA that meets the following conditions:

- Less than 0.005% of genomic DNA by weight
- Free of RT and PCR inhibitors
- Dissolved in PCR–compatible buffer
- Free of RNase activity
- Nondenatured

IMPORTANT! Do not denature the RNA. Denaturation can reduce the yield of cDNA for some gene targets.

Store purified RNA templates at –25°C to –15°C or –86°C to –65°C in nuclease-free water.

RNA template quantification

Template quantification is critical for successful PCR reactions. Measure the absorbance (optical density or O.D.) of a sample at 260 nm (A_{260}) in a spectrophotometer.

Concentration of single-stranded RNA = A_{260} × 40 µg/mL

Note: Absorbance measurements of highly concentrated (O.D.>1.0) or very dilute (O.D.<0.05) RNA samples might be inaccurate. Dilute or concentrate the RNA to obtain a reading within the acceptable range.

Before you begin

- **RNA samples—**Use TE buffer or water for diluting samples.
- **Template quantity—**Use up to 1 µg of RNA template per reaction.

  To determine the optimal template quantity, prepare serial dilutions of the template and test RT–PCR reactions with different template quantities. Select the quantity that produces the earliest C_t without inhibiting PCR.
- **Primer and probe concentrations—**Start with 900 nM forward primer, 900 nM reverse primer, and 250 nM probe in each reaction.

  With custom-designed assays, determine the optimal primer and probe concentrations using the procedures in "Guidelines for custom–designed assays" on page 14.
- **Procedural standards** (for standard curve and relative standard curve experiments)—Standards are critical for accurate analysis of run data. Inaccurate dilutions affect the quality of results.

  The quality of pipettors and tips and the care used in measuring and mixing dilutions affect accuracy.
- **Master mix—**Prior to use, thaw the kit components on ice. Mix the 2X TaqMan™ RT-PCR Mix and 40X TaqMan™ RT Enzyme Mix thoroughly by swirling the bottles.

  After the first use, store the 2X TaqMan™ RT-PCR Mix at 2–8°C to minimize freeze-thaw cycles.

  IMPORTANT! Protect the 2X TaqMan™ RT-PCR Mix from light. Excessive exposure to light might affect the ROX™ dye.
- **Replicates—**Determine the total number of PCR reactions required, including replicates, for each sample.

  We recommend performing at least 3 technical replicates of each reaction.

Prepare the RT–PCR Reaction Mix

Thaw the RT–PCR Mix, RT Enzyme Mix, gene expression assay or primers and probe, and RNA templates on ice. When thawed, gently invert the tubes to mix, then return to the ice.

IMPORTANT! Do not vortex the tubes.

IMPORTANT! Protect the RT–PCR Mix, assays and probe from light until ready for use. Excessive exposure to light might affect the fluorescent dyes.

1. Calculate the volume of components needed based on the reaction volume and the number of reactions, including overage.

   Note: We recommend performing at least 3 technical replicates of each reaction.

2. In microcentrifuge tubes, combine the components as shown in the following tables:

Table 3 TaqMan™ Gene Expression Assays

Component	Volume per reaction[1]		
	10 µL	**20 µL**	**50 µL**
RT-PCR Mix (2X)	5.0 µL	10.0 µL	25.0 µL
Assay (20X)	0.5 µL	1.0 µL	2.5 µL
RT Enzyme Mix (40X)	0.25 µL	0.5 µL	1.25 µL
RNA template (up to 1 µg) + RNase-free water	4.25 µL	8.5 µL	21.25 µL
Total RT–PCR Reaction Mix volume	**10 µL**	**20 µL**	**50 µL**

[1] Add 10% overage to account for pipetting loss.

Table 4 Custom TaqMan™ Gene Expression Assays

Component	Volume per reaction[1]		
	10 µL	**20 µL**	**50 µL**
RT–PCR Mix (2X)	5.0 µL	10.0 µL	25.0 µL
Forward primer (900 nM final)	Variable	Variable	Variable
Reverse primer (900 nM final)	Variable	Variable	Variable
Probe (50–250 nM final)	Variable	Variable	Variable
RT Enzyme Mix (40X)	0.25 µL	0.5 µL	1.25 µL
RNA template (up to 1 µg)	Variable	Variable	Variable
Nuclease-free water	to 10 µL	to 20 µL	to 50 µL
Total RT–PCR Reaction Mix volume	**10 µL**	**20 µL**	**50 µL**

[1] Add 10% overage to account for pipetting loss.

3. Invert the tubes to mix, then centrifuge briefly to collect the contents at the bottoms of the tubes.

4. Pipette the quantity of RT–PCR Reaction Mix shown below into the wells of a reaction plate appropriate for your real-time PCR system:

Plate	RT–PCR Reaction Mix volume
Standard 384-well plate	10 µL
Fast 48-well plate Fast 96-well plate (0.1 mL)	20 µL
Standard 96-well plate	50 µL

5. Seal the reaction plate with optical adhesive film or optical caps, then centrifuge the reaction plate briefly.

   For example, centrifuge at 140 x *g* for 60 seconds.

Set up and run the RT-PCR Reaction Mix plate

Note: See your real-time PCR system's user guide or getting started guide for details on running a quantification experiment.

1. Import the setup file (SDS in TXT format) into the real-time PCR instrument or software.
2. Set the ramp speed and reaction volume appropriate for the reaction plate.
   - Run mode: **Standard**
   - Reaction volume: 10 µL, 20 µL, or 50 µL
3. Set up the thermal protocol.

Step	Temperature	Time	Cycles
Reverse transcription	48°C	15 minutes	1
Enzyme activation	95°C	10 minutes	1
Denature	95°C	15 seconds	40
Anneal/extend	60°C	60 seconds	

4. Load the reaction plate into a real-time PCR instrument.
5. Start the run.

Analyze the results

Examine the plots and data for the experiment results shown below.

- **Standard curve** (standard curve and relative standard curve experiments)—Slope, amplification efficiency, R^2 values, y-intercept, C_t values, and outliers
- **Gene expression plot** (relative standard curve and comparative C_t experiments)—Differences in gene expression and standard deviation in the replicate groups
- **Amplification plots—**Baseline and threshold values and outliers
- **Well table or results table—**C_t values for each well and for each replicate group

Note: For detailed information on evaluating experiment results, see the user guide for your real-time PCR system.

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Guidelines for custom–designed assays

Amplicon site selection

Use the Primer Express™ Software (Cat. No. 4363991) to select an amplicon site within the target sequence. For details on selecting an amplicon site and using the software, see the *Primer Express™ Software Version 3.0 Getting Started Guide* (Pub. No. 4362460).

General amplicon site selection guidelines

Selecting a good amplicon site ensures amplification of the target cDNA without coamplification of the genomic sequence, pseudogenes, and related genes. Follow these guidelines to select good amplicon sites:

- The amplicon should span one or more introns.
  This avoids amplification of the target gene in genomic DNA.
- The primer pair must be specific to the target gene. It must not amplify pseudogenes or other related genes.
- Test the primer pairs, then select the primer pair that produces the highest signal-to-noise ratio.
  The highest signal-to-noise ratio is determined by the earliest C_t with total RNA or mRNA and no amplification with genomic DNA or negative controls.

If the gene does not contain introns

If the gene you are studying does not contain introns, you cannot ensure amplification the target cDNA sequence without coamplification of the genomic sequence. In this case, run control reactions that do not contain reverse transcriptase to determine whether your RNA sample contains DNA. Amplification in the RT– controls indicates that the RNA sample contains DNA. To remove the DNA, treat the RNA sample with DNase I (Cat. No. 18047019).

Probe and primer design

Overview

Using Primer Express™ Software, design a probe to detect amplification of the target sequence, then design primers to amplify the target sequence.

Using Primer Express™ software

Design the probe and primers using Primer Express™ Software as described in the *Primer Express™ Version 3.0 Getting Started Guide* and *Software Help.*

General probe design guidelines

- Keep the GC content in the 20–80% range.
- Avoid runs of identical nucleotides. If repeats cannot be avoided, there must be fewer than 4 consecutive G bases.
- The base at the 5′ end must not be a G.
- Select the strand in which the probe contains more C bases than G bases.
- For singleplex assays, keep the T_m between 68°C to 70°C.

General primer design guidelines

- Choose the primers after the probe.
- Do not overlap primer and probe sequences. The optimal primer length is 20 bases.
- Keep the GC content in the 20–80% range.
- Avoid runs of identical nucleotides. If repeats cannot be avoided, there must be fewer than 4 consecutive G bases.

IMPORTANT! Important: Keep the T_m between 58°C to 60°C.

- Make sure the last 5 nucleotides at the 3′ end contain no more than two G and/or C bases.
- If you cannot find acceptable primer sequences, you may need to examine the sequence and select another amplicon site or screen for more sites.

Calculation of oligonucleotide concentrations

After you receive your primers and probe, use a spectrophotometric method to determine the concentrations of the oligonucleotides in your assay.

Calculate oligonucleotide concentrations

1. Calculate the sum of extinction coefficient contributions for each oligonucleotide sequence:

Chromophore	Extinction Coefficient
A	15,200
C	7050
G	12,010
T	8400
FAM™ dye	20,958
TAMRA™ dye	31,980
TET™ dye	16,255

(continued)

Chromophore	Extinction Coefficient
JOE™ dye	12,000
VIC™ dye	30,100

2. Measure the absorbance at 260 nm (A_{260}) of each oligonucleotide diluted in TE buffer (for example, 1:100).

3. Calculate the oligonucleotide concentration using the formula:

   A_{260} = (sum of extinction coefficient contributions × cuvette pathlength × concentration) ÷ dilution factor

   Rearrange to solve for concentration:

   Concentration (C) = (dilution factor × A_{260}) ÷ (sum of extinction coefficient contributions × cuvette pathlength)

An example calculation of primer concentration

If the primer sequence is CGTACTCGTTCGTGCTGC:

- Sum of extinction coefficient contributions:
  = A × 1 + C × 6 + G × 5 + T × 6
  = 167,950 $M^{-1}cm^{-1}$
- Example A_{260} measurements:
  Dilution = 1:100
  Cuvette pathlength = 0.3 cm
  A_{260} = 0.13
- Primer concentration:
  = (100 × 0.13) ÷ (167,950 $M^{-1}cm^{-1}$ × 0.3 cm)
  = 2.58 × 10^{-4} M
  = 258 µM

An example calculation of probe concentration

If the probe sequence is CGTACTCGTTCGTGCTGC, FAM™ dye is attached to the 5′ end, and TAMRA™ dye is attached to the 3′ end:

- Sum of extinction coefficient contributions:
  = A × 1 + C × 6 + G × 5 + T × 6 + FAM™ × 1 + TAMRA™ × 1
  = 220,888 $M^{-1}cm^{-1}$
- Example A_{260} measurements:
  Dilution = 1:100
  Cuvette pathlength = 0.3 cm
  A_{260} = 0.13
- Probe concentration:
  = (100 × 0.13) ÷ (220,888 $M^{-1}cm^{-1}$ × 0.3 cm)
  = 1.96 × 10^{-4} M
  = 196 µM

Determine optimal primer concentrations

With your custom-designed assay, determine the primer concentrations to use to obtain the earliest threshold cycle (C_T) and the maximum baseline-corrected normalized reporter (ΔR_n).

Primer concentrations

Use the TaqMan™ RT-PCR Mix (2X) to prepare four technical replicates of each of the nine conditions shown below (36 reactions).

Forward primer final concentration	Reverse primer final concentration		
	50 nM	300 nM	900 nM
50 nM	50/50	50/300	50/900
300 nM	300/50	300/300	300/900
900 nM	900/50	900/300	900/900

Prepare and run the RT-PCR reactions

1. Prepare the 36 RT-PCR reactions:

Component	Volume for one reaction		
	10 µL	20 µL	50 µL
TaqMan™ RT-PCR Mix (2X)	5.0 µL	10.0 µL	25.0 µL
Forward primer (50 nM, 300 nM, or 900 nM final)	Variable	Variable	Variable
Reverse primer (50 nM, 300 nM, or 900 nM final)	Variable	Variable	Variable
TaqMan™ probe (250 nM final)	Variable	Variable	Variable

(continued)

Component	Volume for one reaction		
	10 µL	20 µL	50 µL
TaqMan™ RT Enzyme Mix (40X)	0.25 µL	0.5 µL	1.25 µL
RNA template (10 ng to 100 ng)	Variable	Variable	Variable
Nuclease-free water	to 10 µL	to 20 µL	to 50 µL
Total volume	**10 µL**	**20 µL**	**50 µL**

2. Run the RT-PCR reactions:
   - Run mode: **Standard**
   - Reaction volume: 10 µL, 20 µL, or 50 µL
   - Thermal cycling conditions:

Step	Temperature	Time	Cycles
Reverse transcription	48°C	15 minutes	1
Enzyme activation	95°C	10 minutes	1
Denature	95°C	15 seconds	40
Anneal/Extend	60°C	1 minute	

Note: See the appropriate instrument user guide for detailed instructions to program the thermal cycling parameters, or to run the plate.

Evaluate the results

1. Review the ΔR_n values to identify the optimal primer concentrations for PCR yield.
2. Review the C_T values to identify the optimal primer concentrations for C_T and detect any potential nonspecific amplification in the negative controls.
3. Select the forward primer and reverse primer combination that produces the earliest C_T and the highest ΔR_n.

Determine optimal probe concentration

With your custom-designed assay, determine the probe concentration to use to obtain the earliest threshold cycle (C_T) for the target sequence.

Probe concentrations

Use the TaqMan™ RT-PCR Mix (2X) to prepare four replicates of reactions with the following final probe concentrations: 50 nm, 100 nm, 150 nm, 200 nm, and 250 nm (20 reactions). Use the optimal primer concentrations you determined in the experiment you performed in “Determine optimal primer concentrations” on page 17.

Prepare and run the RT-PCR reactions

1. Prepare the 20 RT-PCR reactions:

Component	Volume for one reaction		
	10 µL	20 µL	50 µL
TaqMan™ RT-PCR Mix (2X)	5.0 µL	10.0 µL	25.0 µL
Forward primer (optimal)	Variable	Variable	Variable
Reverse primer (optimal)	Variable	Variable	Variable
TaqMan™ probe (50 nM, 100 nM, 150 nM, 200 nM, or 250 nM final)	Variable	Variable	Variable
TaqMan™ RT Enzyme Mix (40X)	0.25 µL	0.5 µL	1.25 µL
RNA template (10 ng to 100 ng)	Variable	Variable	Variable
Nuclease-free water	to 10 µL	to 20 µL	to 50 µL
Total volume	**10 µL**	**20 µL**	**50 µL**

2. Run the RT-PCR reactions:
   - Ramp speed or mode: **Standard**
   - Reaction volume: 10 µL, 20 µL, or 50 µL
   - Thermal cycling conditions:

Step	Temperature	Time	Cycles
Reverse transcription	48°C	15 minutes	1
Enzyme activation	95°C	10 minutes	1
Denature	95°C	15 seconds	40
Anneal/Extend	60°C	1 minute	

Evaluate the results

1. Review the ΔR_n values to identify the optimal probe concentration for PCR yield.

2. Review the C_T values to identify the optimal probe concentration for C_T and detect any potential nonspecific amplification in the negative controls.

3. Select the probe concentration that produces the earliest C_T and the highest ΔR_n.

PCR good laboratory practices

Overview

PCR assays require special laboratory practices to avoid false positive amplifications (Kwok and Higuchi, 1989). The high throughput and repetition of PCR assays can lead to amplification of a single DNA molecule (Saiki *et al.*, 1985; Mullis and Faloona, 1987).

About AmpliTaq Gold™ DNA polymerase, UP

The TaqMan™ RT-PCR Mix (2X) contains AmpliTaq Gold™ DNA Polymerase, UP (Ultra Pure), a highly purified DNA polymerase that allows an automated and efficient hot start PCR. The enzyme is modified to be inactive until heat-activated.

The high-temperature incubation step required for activation ensures that the enzyme is activated only at temperatures where the DNA is fully denatured.

When AmpliTaq Gold™ DNA Polymerase, UP is added to the reaction mixture at room temperature, the inactive enzyme is not capable of primer extension. Therefore, any low-stringency mispriming events that may have occurred are not enzymatically extended and subsequently amplified.

About UDG

Uracil-DNA glycosylase (UDG), also known as uracil-N-glycosylase (UNG), hydrolyzes single- or double-stranded DNA that contains dUMP.

UDG treatment can prevent the reamplification of carryover PCR products by removing any uracil incorporated into amplicons (Longo *et al.*, 1990). UDG treatment is effective only if all previous PCR for that assay was performed using a master mix that contains dUTP.

UDG is not compatible with reverse transcription when dUTP is available because UDG will degrade the resulting cDNA. Therefore, UDG should not be used during one-step RT-PCR. However, the TaqMan™ RNA-to-CT™ *1-Step*™ Kit contains a dUTP/dTTP blend so that amplicons produced by the kit are degradable by UDG in subsequent PCR reactions.

General RNA handling practices

When working with RNA, prevent RNase contamination:

- Wear clean gloves throughout the experiment to prevent contamination from RNases on your hands.
- Change gloves frequently, including after touching skin, door knobs, and common surfaces.
- Maintain an RNase-free area in the lab and use dedicated RNase-free equipment and supplies:
  - Use tips and tubes that are tested and guaranteed RNase-free.
  - Use RNase-free chemicals and reagents.
  - Work in a low-traffic area that is away or shielded from air vents or open windows.
  - Clean laboratory surfaces, such as benchtops, centrifuges, and electrophoresis equipment, with RNase*Zap*™ or a mild solution of bleach or sodium hydroxide.

General PCR practices

When preparing PCR reactions:

- Wear a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation) and clean gloves.
- Change gloves whenever you suspect that they are contaminated.

- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Centrifuge tubes briefly before opening them. Open and close all sample tubes carefully.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution.

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.

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.

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.

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

Related documentation

Document	Pub. No.
TaqMan™ RNA-to-C_T™ 1-Step Kit Quick Reference Guide	4393464
TaqMan™ RNA-to-C_T™ 1-Step Kit Product Information Sheet	4392668

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  - 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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