

TaqMan™ RNA-to-C_T™ 1-Step Kit

Catalog Numbers 4392653, 4392938, and 4392656

Pub. No. 4392668 Rev. C

Note: For safety and biohazard guidelines, see the “Safety” appendix in the following product documentation: *TaqMan™ RNA-to-C_T™ 1-Step Kit User Guide* (Pub. No. 4393463). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

Use the TaqMan™ RNA-to-C_T™ 1-Step Kit to perform one step RT-PCR with TaqMan™ reagents for quantification experiments on a real-time PCR system.

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.

Methods

Before you begin

- Thoroughly mix the 2X TaqMan™ RT-PCR Mix. Do not create excess bubbles.
- Thoroughly mix the 40X TaqMan™ RT Enzyme Mix, then briefly centrifuge to resuspend. Do not create excess bubbles.
- Determine the total number of RT-PCR reactions required. We recommend performing four replicates of each reaction.

Prepare the RT-PCR Reaction Mix

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

Component	Volume per reaction		
	384-well plate	96-well (0.1-mL) plate	96-well (0.2-mL) plate
2X TaqMan™ RT-PCR Mix	5 µL	10 µL	25 µL
40X TaqMan™ RT Enzyme Mix	0.25 µL	0.5 µL	1.25 µL
20X TaqMan™ Gene Expression Assay	0.5 µL	1 µL	2.5 µL
RNA template	Variable	Variable	Variable
Nuclease-free water	Variable	Variable	Variable
Total RT-PCR Reaction Mix volume per reaction	10 µL	20 µL	50 µL

2. Vortex briefly to mix.
3. Centrifuge the tubes briefly to spin down the contents and eliminate any air bubbles.

Prepare the RT-PCR reaction plate

1. Transfer the appropriate volume of RT-PCR Reaction Mix to each well of the plate.
 - 384-well plate: 10 μ L
 - 96-well 0.1-mL plate: 20 μ L
 - 96-well 0.2-mL plate: 50 μ L
2. Seal the reaction plate, then centrifuge briefly to bring the RT-PCR Reaction Mix to the bottom of the wells and eliminate air bubbles.

Run the RT-PCR reactions

See the appropriate instrument user guide for detailed instructions to program the thermal-cycling conditions or to run the plate.

1. Set up a plate document or experiment file using the following conditions:

Instrument	Step	Temperature	Time	Cycles
<ul style="list-style-type: none">• StepOne™ Real-Time PCR System• 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 Real-Time PCR System• 7500 Fast Real-Time PCR System• 7900HT Real-Time PCR System	Reverse transcription	48°C	15 minutes	1
	Enzyme activation	95°C	10 minutes	1
	Denaturation	95°C	15 seconds	40
	Annealing/extension	60°C	1 minute	

2. Select Standard cycling mode.

IMPORTANT! TaqMan™ RNA-to-C_T™ 1-Step Kit does not support the fast cycling mode. Use standard cycling mode to run the RT-PCR reactions.

3. Enter the sample volume.
4. Load the reaction plate.
5. Start the run.

Guidelines for data analysis

Data analysis varies depending on the instrument used. Refer to the *TaqMan™ RNA-to-C_T™ 1-Step Kit User Guide* (Pub. No. 4393463) and your instrument documentation for detailed information on data analysis.

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.



Revision history: Pub. No. 4392668

Revision	Date	Description
C	22 February 2023	<ul style="list-style-type: none">The storage conditions for the 2X TaqMan™ RT-PCR Mix and the 40X TaqMan™ RT Enzyme Mix were updated.The volumes per reaction for the RT-PCR Reaction Mix were updated.The real-time PCR instrument list was updated.
B	15 October 2018	Updated for manufacturer, general style, formatting, and branding.
A	26 September 2007	New document.

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.

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.

©2023 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a trademark of Roche Molecular Systems, Inc., used under permission and license.