

# TaqMan™ RNA-to-C<sub>T</sub>™ 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<sub>T</sub>™ 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<sub>T</sub>™ 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 <sup>[1]</sup>
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

<sup>[1]</sup> 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
<b>Total RT-PCR Reaction Mix volume per reaction</b>	<b>10 µL</b>	<b>20 µL</b>	<b>50 µL</b>

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  $\mu$ L
  - 96-well 0.1-mL plate: 20  $\mu$ L
  - 96-well 0.2-mL plate: 50  $\mu$ 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"><li>• StepOne™ Real-Time PCR System</li><li>• StepOnePlus™ Real-Time PCR System</li><li>• QuantStudio™ 3 or 5 Real-Time PCR System</li><li>• QuantStudio™ 6 or 7 Flex Real-Time PCR System</li><li>• QuantStudio™ 6 Pro or 7 Pro Real-Time PCR System</li><li>• QuantStudio™ 12K Flex Real-Time PCR System</li><li>• 7500 Real-Time PCR System</li><li>• 7500 Fast Real-Time PCR System</li><li>• 7900HT Real-Time PCR System</li></ul>	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.

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**IMPORTANT!** TaqMan™ RNA-to-C<sub>T</sub>™ 1-Step Kit does not support the fast cycling mode. Use standard cycling mode to run the RT-PCR reactions.

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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<sub>T</sub>™ 1-Step Kit User Guide* (Pub. No. 4393463) and your instrument documentation for detailed information on data analysis.

## Limited product warranty

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**Revision history:** Pub. No. 4392668

Revision	Date	Description
C	22 February 2023	<ul style="list-style-type: none"><li>• The storage conditions for the 2X TaqMan™ RT-PCR Mix and the 40X TaqMan™ RT Enzyme Mix were updated.</li><li>• The volumes per reaction for the RT-PCR Reaction Mix were updated.</li><li>• The real-time PCR instrument list was updated.</li></ul>
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.

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