Power SYBR™ Green RNA-to-C_T™ *1-Step* Kit

Catalog Numbers 4391178, 4389986

Pub. No. MAN0019838 Rev. A.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Power SYBR™ Green RNA-to-C_T™ 1-Step Kit User Guide* (Pub. No. 4391003). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

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

Contents and storage

Contents	Cat. No. 4391178 (40 × 50-µL reactions)	Cat. No. 4389986 (200 × 50-µL reactions)	Storage[1]
2X *Power* SYBR™ Green RT-PCR Mix	1 mL	5 mL	2–8°C
125X RT Enzyme Mix	20 µL	80 µL	–30°C to –15°C

[1] See packaging for expiration date.

Methods

Before you begin

- Thoroughly mix the 2X *Power* SYBR™ Green RT-PCR Mix. Do not create excess bubbles.
- Thoroughly mix the 125X RT Enzyme Mix, then briefly centrifuge to resuspend. Do not create excess bubbles.
- Determine the total number of PCR reactions required. We recommend performing four replicates of each reaction.

Prepare the 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 *Power* SYBR™ Green RT-PCR Mix	5 µL	10 µL	25 µL
125X RT Enzyme Mix	0.08 µL	0.16 µL	0.4 µL
Forward Primer (100–200 nM final concentration)	Variable	Variable	Variable
Reverse Primer (100–200 nM final concentration)	Variable	Variable	Variable
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 briefly to bring the PCR Reaction Mix to the bottom of the tube and eliminate air bubbles.

Prepare the PCR reaction plate

1. Transfer the appropriate volume of PCR Reaction Mix to each well of the plate.
   - 384-well plate: 10 µL
   - 96-well 0.1-mL plate: 20 µL
   - 96-well 0.2-mL plate: 50 µL
2. Seal the reaction plate, then centrifuge briefly to bring the 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	Duration	Cycles
• QuantStudio™ 5 Real-Time PCR System • QuantStudio™ 6 or 7 Flex 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	30 minutes	Hold
	Enzyme activation	95°C	10 minutes	Hold
	Denaturation	95°C	15 seconds	40
	Annealing/extension	60°C	1 minute	

2. Select Standard cycling mode.

   IMPORTANT! *Power* SYBR™ Green 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 *Power SYBR™ Green RNA-to-C_T™ 1-Step Kit User Guide* (Pub. No. 4391003) and your instrument documentation for detailed information on data analysis.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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Revision history: Pub. No. MAN0019838

Revision	Date	Description
A.0	4 December 2020	Replaces Pub. No. 4391588, Rev. B. The following edits are included in MAN0019838 Rev. A.0: • Corrected volumes to prepare RT-PCR Reaction Mix. • Updated the real-time PCR instruments.