High-Capacity RNA-to-cDNA™ Kit

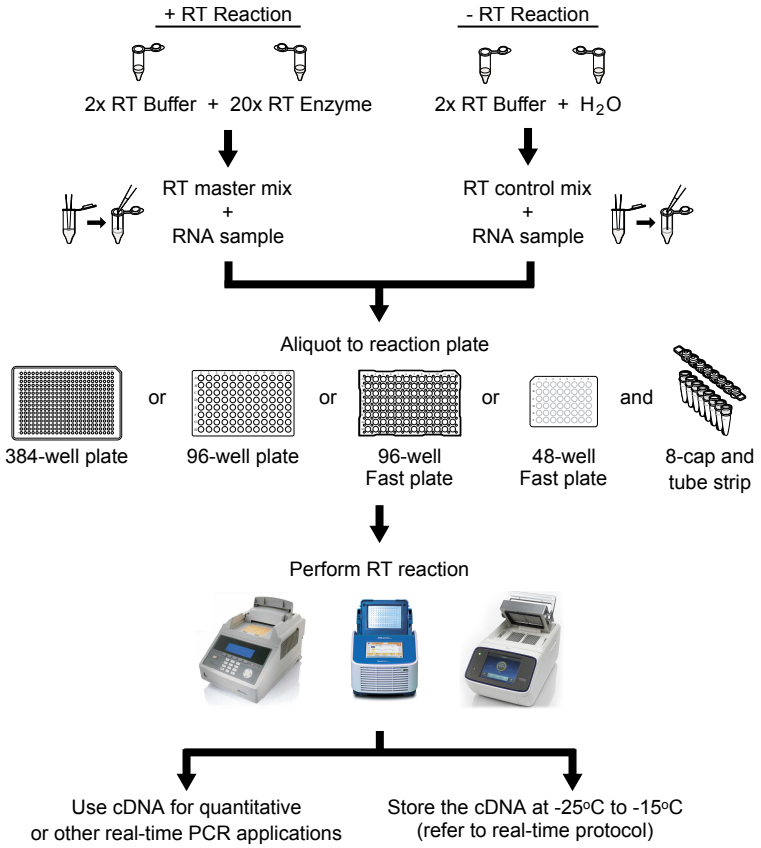
Catalog Number 4387406

Pub. No. 4392339 **Rev.** C

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *High-Capacity RNA-to-cDNA™ Kit User Guide* (Pub. No. 4387951). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Workflow

To synthesize single-stranded cDNA from total RNA using the Applied Biosystems™ High-Capacity RNA-to-cDNA™ Kit:



Guidelines for RNA

Use up to 2 µg of total RNA per 20-µL reaction.

For optimal performance of the kit, we recommend that you use RNA that is:

- Free of inhibitors of reverse transcription and PCR
- Dissolved in TE Buffer or PCR-compatible buffer
- Free of RNase activity

Methods

1 Prepare the RT reaction mix

Prepare the RT reaction mix (per 20-µL reaction) using the kit components before preparing the reaction plate:

1. Allow the kit components to thaw on ice.
2. Calculate the volume of components needed to prepare the required number of reactions.

Component	Volume per reaction	
	+RT reaction	–RT reaction
2X RT Buffer Mix	10.0 µL	10.0 µL
20X RT Enzyme Mix	1.0 µL	—
RNA sample	up to 9 µL	up to 9 µL
Nuclease-free H_2O	Q.S.[1] to 20 µL	Q.S.[1] to 20 µL
Total per reaction	20.0 µL	20.0 µL

[1] Quantity Sufficient

Note: Prepare the RT reaction mix on ice. Include additional reactions in the calculations to provide excess volume for the loss that occurs during reagent transfers.

2 Prepare the reverse transcription reactions

a. Prepare the reaction plate or tubes:
   1. Aliquot 20 µL of RT reaction mix into each well or tube.
   2. Seal the plates or tubes.
   3. Briefly centrifuge the plate or tubes to spin down the contents and to eliminate any air bubbles.

b. Place the plate or tubes on ice until you are ready to load the thermal cycler or Applied Biosystems™ Real-Time PCR system.

3 Perform reverse transcription

a. Using one of the required thermal cyclers listed in the *High-Capacity RNA-to-cDNA™ Kit User Guide* (Pub. No. 4387951), program the thermal cycling conditions:

IMPORTANT! These conditions are optimized for use with the High-Capacity RNA-to-cDNA™ Kit.

Setting	Step 1	Step 2	Step 3
Temperature	37°C	95°C	4°C
Time	60 minutes	5 minutes	∞

b. Set the reaction volume to 20 µL.

c. Load the reactions into the thermal cycler or Real-Time PCR system.

d. Start the reverse transcription run.

4 Store the cDNA

a. Store cDNA RT plates or tubes prepared using the High-Capacity RNA-to-cDNA™ Kit for short-term or long-term storage:
   - Short-term (up to 24 hours before use)—Store at 2–8°C.
   - Long-term—Store at –25°C to –15°C.

b. If required, briefly centrifuge the archive plates or tubes before storing to spin down the contents and to eliminate any air bubbles.

Limited product warranty

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Revision history. Pub. No. 4392339

Revision	Date	Description
C	2 August 2016	Format, style, and legal updates
B	23 May 2016	Format update
A	September 2007	Baseline for this revision history