

High-Capacity cDNA Reverse Transcription Kits

Catalog Numbers 4368813, 4368814, 4374966, and 4374967

Pub. No. 4375222 Rev. C

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit uses the random primer scheme for initiating cDNA synthesis. Random primers ensure that the first strand synthesis occurs efficiently with all species of RNA molecules present, including mRNA and rRNA. The kit has been tested extensively and validated against various RNA templates, including G/C-rich and A/U-rich RNA species. The effect of relative mRNA abundance was also examined. An essential requirement for the relative quantitation of cDNA is that the reverse transcriptase reaction generates products in a manner directly dependent on the amount of input RNA template. In all cases, quantitative conversion of mRNA and 18S ribosomal RNA species was observed.

Available kits

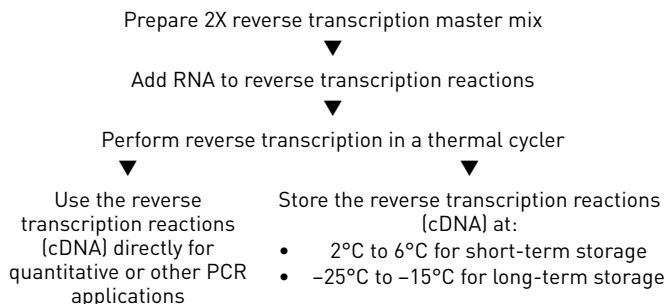
| Kit | Cat. No. |
|---|----------|
| High-Capacity cDNA Reverse Transcription Kit, 200 reactions | 4368814 |
| High-Capacity cDNA Reverse Transcription Kit, 1000 reactions | 4368813 |
| High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor, 200 reactions | 4374966 |
| High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor, 1000 reactions | 4374967 |

Contents and storage

| Contents | Cat. Nos. 4368813 and 4374967 | Cat. Nos. 4368814 and 4374966 | Storage |
|---|-------------------------------|-------------------------------|----------------|
| 10X RT Buffer, 1.0 mL | 2 tubes | 1 tube | -25°C to -15°C |
| 10X RT Random Primers, 1.0 mL | 2 tubes | 1 tube | |
| 25X dNTP Mix (100 mM) | 1 tube, 1.0 mL | 1 tube, 0.2 mL | |
| MultiScribe™ Reverse Transcriptase, 50 U/μL | 1 tube, 1.0 mL | 2 tubes, 0.1 mL | |
| RNase Inhibitor, 100 μL ^[1] | 10 tubes | 2 tubes | |

^[1] Included in Cat. Nos. 4374966 and 4374967 only.

Workflow



Reverse transcription reaction guidelines

The kit contains reagents that, when combined, form a 2X reverse transcription (RT) master mix. An equal volume of RNA sample should be added. To avoid RNase contamination, RNase-free reagents and consumables must be used.

Prepare the 2X RT master mix

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

Note: Prepare the RT master mix on ice.

| Component | Volume | |
|------------------------------------|----------------------|-------------------------|
| | With RNase Inhibitor | Without RNase Inhibitor |
| 10X RT Buffer | 2.0 μL | 2.0 μL |
| 25X dNTP Mix (100 mM) | 0.8 μL | 0.8 μL |
| 10X RT Random Primers | 2.0 μL | 2.0 μL |
| MultiScribe™ Reverse Transcriptase | 1.0 μL | 1.0 μL |
| RNase Inhibitor | 1.0 μL | — |
| Nuclease-free H ₂ O | 3.2 μL | 4.2 μL |
| Total per reaction | 10.0 μL | 10.0 μL |

IMPORTANT! Include additional reactions in the calculations to provide excess volume for the loss that occurs during reagent transfers.

3. Place the 2X RT master mix on ice and mix gently.

Prepare the reverse transcription reactions

1. Pipette 10 μ L of 2X RT master mix into each well of a 96-well reaction plate or individual tube.
2. Pipette 10 μ L of RNA sample into each well, pipetting up and down two times to mix.
3. Seal the plates or tubes.
4. Briefly centrifuge the plate or tubes to spin down the contents and to eliminate any air bubbles.
5. Place the plate or tubes on ice until you are ready to load the thermal cycler.

Program the thermal cycling conditions

Program the thermal cycler using the conditions below.

IMPORTANT! These conditions are optimized for use with the High-Capacity cDNA Reverse Transcription Kits.

| Settings | Step 1 | Step 2 | Step 3 | Step 4 |
|----------|------------|-------------|-----------|----------|
| Temp. | 25°C | 37°C | 85°C | 4°C |
| Time | 10 minutes | 120 minutes | 5 minutes | ∞ |

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

DISCLAIMER

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Table 1 Revision history of Pub. No. 4375222

| Revision | Date | Description |
|----------|---------------|------------------------------------|
| C | 21 March 2016 | Format, style, and legal updates |
| B | 13 April 2006 | Baseline for this revision history |

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