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Working

cDNA synthesis using the Applied BiosystemsTM High-Capacity cDNA Reverse Transcription Kit

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ABSTRACT

cDNA synthesis using the Applied Biosystems^{TM}High-Capacity cDNA Reverse Transcription Kit

MATERIALS

NAME	CATALOG #	VENDOR
Applied BiosystemsTM High-Capacity cDNA Reverse Transcription Kit	4368814	Applied Biosystems

1 Allow the kit components to thaw on ice.
Prepare the 2X RT master mix on ice.

Component	Volume (µL)
10X RT Buffer	2.0
25X dNTP Mix (100 mM)	0.8
10X RT Random Primers	2.0
MultiScribe^{TM}Reverse Transcriptase	1.0
RNase Inhibitor	1.0
Nuclease-free H_2O	3.2
Total per reaction	10.0

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

2 Pipette 10 µL of 2X RT master mix into individual tube.

3 Pipette 10 µL of RNA sample (800 - 1000ng total RNA) into each tube, pipetting up and down two times to mix.
Seal the plates or tubes.

4 Briefly centrifuge the tubes to spin down the contents and to eliminate any air bubbles.

5 Place the tubes on ice until you are ready to load the thermal cycler.

6 Program the thermal cycler using the conditions below.

Settings	Step 1	Step 2	Step 3	Step 4
Temperature	25 °C	37 °C	85 °C	4 °C
Time	10 minutes	120 minutes	5 minutes	∞