

May 15, 2019

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

 [PLOS One](#)

DOI

dx.doi.org/10.17504/protocols.io.2v6ge9e

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OPEN  ACCESS



DOI: dx.doi.org/10.17504/protocols.io.2v6ge9e

External link: <https://doi.org/10.1371/journal.pone.0226005>

Protocol Citation: Lisa-Maria Rosenthal, Giang Tong, Katharina Schmitt 2019. cDNA synthesis using the Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit. [protocols.io](https://dx.doi.org/10.17504/protocols.io.2v6ge9e) <https://dx.doi.org/10.17504/protocols.io.2v6ge9e>

Manuscript citation:

Rosenthal L, Leithner C, Tong G, Streitberger KJ, Krech J, Storm C, Schmitt KRL (2019) RBM3 and CIRP expressions in targeted temperature management treated cardiac arrest patients—A prospective single center study. PLoS ONE 14(12): e0226005. doi: [10.1371/journal.pone.0226005](https://doi.org/10.1371/journal.pone.0226005)

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Protocol status: Working

We use this protocol and it's working

Created: May 15, 2019

Last Modified: May 15, 2019

Protocol Integer ID: 23198



Abstract

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Materials

MATERIALS



Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit **Applied Biosystems (ThermoFisher Scientific) Catalog #4368814**



- 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™ Reverse Transcriptase	1.0
RNase Inhibitor	1.0
Nuclease-free H ₂ O	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	∞