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 We use this protocol and it's working

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First strand cDNA synthesis (ThermoScientific RevertAid)

Forked from [First strand cDNA synthesis](#)

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ABSTRACT

The following protocol is optimized to generate first-strand cDNA for use in two step-PCR.

MATERIALS

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⊗ Maxima H Minus Reverse Transcriptase **Thermo Fisher Scientific Catalog ##EP0741**

⊗ 5X RT Buffer **Thermo Fisher Scientific Catalog ##B91**

⊗ Random Hexamer **Thermo Fisher Scientific Catalog ##S0142**

⊗ dNTP Mix 10 mM each **Thermo Fisher Scientific Catalog ##R0191**

⊗ Water, nuclease free **Contributed by users**

⊗ RiboLock RNase Inhibitor **Thermo Fisher Scientific Catalog ##E00381**

BEFORE START INSTRUCTIONS

Mix and briefly centrifuge all reagents after thawing, keep on ice.

1 Add reaction components into sterile, nuclease-free tube on ice in the indicated order:

Template RNA	100 ng (1pg - 5 µg)
Random Hexamer	1 µl (100 pmol)
Water, nuclease-free	to 13.5 µl

- 2 Optional:** If the RNA template is GC-rich or is known to contain secondary structures, mix gently, centrifuge briefly and incubate at 65 °C for 5min. Chill on ice, briefly centrifuge again and place on ice.

3

5X RT Buffer	4 µl
RiboLock RNase Inhibitor	0.5 µl (20 U)
Maxima Reverse Transcriptase	1 µl (200 U)
dNTP Mix	1 µl
Total volume	20 µl

Mix gently and centrifuge briefly.

4

10 min	25 °C
60 min	42 °C (For GC-rich RNA, the reaction temperature can be increased to 45 °C)
10 min	70 °C

- 5** Add to 80 µl nuclease-free Water.

Can be used directly in qPCR or stored at -20 °C for up to one week.