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First strand cDNA synthesis (ThermoScientific RevertAid)

Forked from [First strand cDNA synthesis \(ThermoScientific RevertAid\)](#)

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We use this protocol and it's working

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ABSTRACT

The following protocol is optimized to generate first-strand cDNA for use in (q)PCR

MATERIALS

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- ⊗ 5X RT Buffer **Thermo Fisher Scientific Catalog ##B91**
- ⊗ dNTP Mix 10 mM each **Thermo Fisher Scientific Catalog ##R0191**
- ⊗ Water, nuclease free **Contributed by users**
- ⊗ RiboLock RNase Inhibitor **Thermo Fisher Scientific Catalog ##E00381**
- ⊗ RevertAid Reverse Transcriptase (200 U/μL) **Thermo Fisher Catalog #EP0441**
- ⊗ Oligo(dT)18 Primer **Thermo Fisher Catalog #S0131**

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)
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Oligo(dT)18	1 µl (100 pmol)
Water, nuclease-free	to 12 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	1 µl (20 U)
RevertAid RT (200 U/µL)	2 µl (400 U)
10 mM dNTP Mix	1 µl
Total volume	20 µl

Mix gently and centrifuge briefly.

4

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

- 5** The cDNA product is now ready for downstream applications