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First strand cDNA synthesis (ThermoScientific RevertAid)

Forked from First strand cDNA synthesis (ThermoScientific RevertAid)

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1 Works for me dx.doi.org/10.17504/protocols.io.8bmhsk6

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ABSTRACT

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

MATERIALS

NAME	CATALOG #	VENDOR
5X RT Buffer	#B91	Thermo Fisher Scientific
dNTP Mix 10 mM each	#R0191	Thermo Fisher Scientific
Water, nuclease free		
RiboLock RNase Inhibitor	#EO0381	Thermo Fisher Scientific
RevertAid Reverse Transcriptase (200 U/µL)	EP0442	Thermo Fisher
Oligo(dT)18 Primer	SO131	Thermo Fisher

BEFORE STARTING

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)
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
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