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Working

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.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Maxima H Minus Reverse Transcriptase	#EP0741	Thermo Fisher Scientific
5X RT Buffer	#B91	Thermo Fisher Scientific
Random Hexamer	#SO142	Thermo Fisher Scientific
dNTP Mix 10 mM each	#R0191	Thermo Fisher Scientific
Water, nuclease free		
RiboLock RNase Inhibitor	#EO0381	Thermo Fisher Scientific

SAFETY WARNINGS

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)
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
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.