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First strand cDNA synthesis (ThermoScientific RevertAid)

Forked from First strand cDNA synthesis

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Protocol status: Working
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 two step-PCR.

Materials

MATERIALS

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

Before start

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.