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.

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Before start

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

Materials

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

Protocol

Step 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)
dNTP Mix	1 µl (final concentration)
Water, nuclease-free	**to 14.5 µl**

Step 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

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Step 3.

Add the following reaction components in the indicated order:

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

Mix gently and centrifuge briefly.

Step 4.

Incubate:

10 min	25 °C
30 min	50 °C (For GC-rich RNA, the reaction temperature can be increased to 65 °C)
5 min	85 °C

Step 5.

Add to 80 µl nuclease-free Water**.**

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