

Mar 22, 2019

First strand cDNA synthesis (ThermoScientific RevertAid)



Forked from [First strand cDNA synthesis](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.ze3f3gn>

Sebastian Triesch¹, Maximilian Dietsch¹

¹Institute for Synthetic Microbiology, HHU Düsseldorf

Axmann Lab

CyanoWorld

1 more workspace



Sebastian ST Triesch

Institute for Synthetic Microbiology, HHU Düsseldorf

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.ze3f3gn>

Protocol Citation: Sebastian Triesch, Maximilian Dietsch 2019. First strand cDNA synthesis (ThermoScientific RevertAid). protocols.io <https://dx.doi.org/10.17504/protocols.io.ze3f3gn>

License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited



Protocol status: Working

We use this protocol and it's working

Created: March 22, 2019

Last Modified: March 22, 2019

Protocol Integer ID: 21691

Keywords: cDNA synthesis, RT-PCR, RT-qPCR, first strand cdna synthesis, strand cdna for use, strand cdna, thermoscientific revertaid, pcr

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.

- 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 μ l nuclease-free Water.

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