OCT 14, 2019

Reverse transcription of RNA to cDNA

Igem Dusseldorf¹

¹Heinrich-Heine Universität Düsseldorf

Igem Dusseldorf

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.77chriw

Protocol Citation: Igem Dusseldorf 2019. Reverse transcription of RNA to cDNA.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.77chriw>

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: Oct 14, 2019

Last Modified: Oct 14, 2019

PROTOCOL integer ID:

28612

ABSTRACT

- 100 ng RNA
- 40x yellow sample buffer
- random hexamer primers
- 5x RT-buffer
- dNTP Mix (10 mM of each nucleotide)
- RevertAid Reverse Transcriptase
- *You will require 100 ng for RT and 100 ng for -RT control (include a -RT control for each sample!)*

Preparation of 1.33X yellow buffer

33,25 µL 40x yellow sample buffer + 966,75 µL H₂O

Start

In PCR stripes, pipet in the following order:

100 ng template RNA

1 µL random hexamer primers

RNase-free H₂O to 12,5 µL volume

Prepare a Mastermix of the following reagents:

4 µL 5x RT-buffer

0.5 µL RiboLock RNase-Inhibitor

2 µL dNTP Mix (10 mM of each nucleotide)

1 µL RevertAid Reverse Transcriptase

Add 7.5 µL Mastermix to each reaction

For your -RT control, pipet 100 ng RNA in PCR stripes, leave out buffer and everything, just add H₂O to a final volume of 20 µL. Mark as -RT so you can distinguish it from your actual cDNA!

PCR Programm

10 min 25 °C

60 min 42 °C

10 min 70 °C

After completion of RT reaction:

Add 60 µL 1,33 x yellow sample buffer to each reaction

cDNA can be stored at 4 °C for a short time, otherwise, freeze at -20 °C