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Reverse transcription of RNA to cDNA

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Works for me

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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



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