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Protocol status: Working

RNA to cDNA and RT-PCR protocol

PLOS One

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ABSTRACT

Used applied biosystems high-capacity RNA-to-cDNA kit and fast SYBR green master mix protocol

GUIDELINES

Use up to 2 µg of total RNA per 20-µL reaction

MATERIALS

RNA-to-cDNA:
 2X RT buffer
 2X RT enzyme
 RNA sample
 384 -or 96-well plate
 8-cap and tube strip

RT-PCR:
 Fast SYBR Green Master Mix
 cDNA samples
 Primers
 RNase-free water
 7900HT PCR instrument

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RNA to cDNA reverse transcription

- 1 Prepare the RT reaction mix
2X buffer mix : 10 μL
20X RT enzyme Mix: 1 μL
RNA sample: < 9 μL
Nuclease-free water: quantity sufficient to 20 μL
Total per reaction: 20 μL
- 2 Prepare the reverse transcription reactions:
Aliquot 20 μL RT reaction mix into each well
Seal the plates and centrifuge to spin down the contents and remove air bubbles
Store them on ice
- 3 Perform reverse transcription:
Step1:
Temp. 37 °C , time: 60 mins
Step2:
Temp. 95 °C , time: 5 mins
Step3:
Temp. 4 °C , time: unlimited

Store the cDNA:
Short-term: < 24 hours before use and store at 2–8°C
Long-term: store at –25°C to –15°C

Prepare PCR reaction plate

- 4 For one reaction:
Fast SYBR® Green Master Mix (2×) : 10 μL
Forward and Reverse Primers : variable
cDNA template + RNase-free water: variable

Total: 20 µL

Mix by gentle inversion, then centrifuge the tube to spin down the contents and eliminate any air bubbles

- 5 Transfer the appropriate volume of each reaction to 384-well reaction plate

Run the PCR reaction plate

- 6 Instrument: 7900HT

AmpliTaQ Fast DNA Polymerase, UP Activation :

Temp. 95 °C , time 10 mins, Cycles HOLD

Denature :

Temp. 95 °C , time 1second

Temp. 60 °C , time 20 seconds

Anneal/extend:

Temp. 95 °C , time 15 seconds

Temp. 60 °C , time 15 seconds

Temp. 95 °C , time 15 seconds

Reaction volume: 20 µL

MicroAmp Optical 384-Well Reaction Plate

- 7 Analyze the data