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qPCR

In 2 collections

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We use this protocol and it's working

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Abstract

Standard protocol for RNA extraction and qPCR

RNA Extraction

1 Wash cells with PBS

2 Lyse cells in TRIzol (Invitrogen 15596018). For 1 well of a 6-well plate, use 300 uL of TRIzol

3 Freeze TRIzol lysate or continue to the next step

4 Use an RNA extraction kit, following manufacturer's recommendation (eg Zymo R2062)

5 Measure extracted RNA concentration and purity on a spectrophotometer

6 Store RNA at -80°C

cDNA

7 Thaw RNA on ice if not continuing directly from step 5

8 Prepare the reverse transcription master mix as follows: 2X RT buffer (Thermo Scientific EP0752), 1mM each dNTP mix (Thermo Scientific #R0191), 10uM random hexamers (Thermo Scientific #SO142), 50 U Maxima H Minus Reverse Transcriptase per reaction(Thermo Scientific EP0752), and nuclease free water up to 10uL per reaction.

9 In a PCR tube, add 1 ug of RNA and nuclease free water up to 10 uL. The amount of RNA can be lowered if RNA concentration is low.

10 Add 10 uL of the master mix from step 8 to each sample

11 Incubate for 10 minutes at 25°C

12 Incubate for 30 minutes at 50°C

13 Store cDNA at -20°C

qPCR

14 Thaw cDNA on ice if not continuing directly from step 12

15 Dilute cDNA 1:10 in nuclease free water

16 Prepare primer mixes with 10uM of forward primer and 10uM of reverse primer in nuclease free water

17 In each well of a 384 well plate, combine 2X PowerUp SYBR Green Master Mix (Applied Biosystems A25741), 1uM each of forward and reverse primers, and diluted cDNA to a final volume of 10uL.

18 Run qPCR with the following cycle:

18.1 2 minutes at 50°C

18.2 10 minutes at 95°C

18.3 40 cycles of 15 seconds at 95°C and 1 minute at 60°C

18.4 Optionally, include a melting curve stage: 15 seconds at °C, 1 minute at 60°C and a gradual increase of 0.05°C/second back to 95°C

19 Analyze using the ΔΔCt method