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RNA isolation and qRT-PCR

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We use this protocol and it's working

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Abstract

This protocol describes the RNA isolation from cultured cells and quantitative RT PCR.

Attachments

DOCX

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136KB

Materials

Reagents require:

1. RNeasy Micro Plus kit (Qiagen)
2. iScript cDNA synthesis Kit (Bio-Rad)
3. Primers
4. SYBR Green Master Mix (BioRad)
5. 96 well plates (BioRad)
6. Optical Adhesive Covers

RNA isolation and qRT-PCR

1 Aspirate media from cells and rinse cells with PBS On ice .

2 Isolate RNA using RNeasy Micro Plus kit (Qiagen) according to manufacturer's protocol.

3 Generate cDNA from 1 µg purified RNA using iScript cDNA synthesis Kit (Bio-Rad) according to manufacturer's protocol.

4 The iScript cDNA was diluted 1:10 by using sterile water.

5 Combine 10 µL SYBR Green Master Mix (BioRad) with 6.78 µL Sterile Water per sample.

6 Combine 16.78 µL diluted SYBR Green Master Mix with 0.61 µL each of [M] 10 micromolar (µM) forward and reverse primers per sample. Pipette this mixture into wells of 96-well qPCR plate 2 technical replicates were ran for each sample.

7 Pipette 2 µL of diluted RNA from step 4 in well with SYBR Green Master Mix.

8 Cover plate with Optical Adhesive Covers (Applied Biosystems).

9 Spin down plate in tabletop centrifuge.

10 Run qPCR in CFX96 Real-Time System (BioRad) using the following PCR steps.

11 PCR cycle.

temperature	time	
95	5 minutes	
95	15 seconds	39 cycles
60	30 seconds	
75	10 seconds	Melt curve
95	20 seconds	

Analysis of qRT PCR data

12 Calculate the ΔCT values by subtracting respective gene CT value from housekeeping gene GAPDH value.

13 Followed by calculating $2^{\Delta CT}$ values, normalize the mRNA expression levels for genes of interest to GAPDH and represented relative to control.