Total RNA extraction, cDNA synthesis, and qPCR(S100A6-siRNA)

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Abstract

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Protocol

Step 1.
Wash the cells in a Petri dish. After washing twice with 1 × PBS, add 1 ml of Trizol solution and mix well and pipette into 1.5 ml RNase free EP tube to lyse the cells thoroughly for 5 minutes at room temperature.

Step 2.
Add 200 µL of chloroform to the centrifuge tube and shake vigorously for 30s. centrifuge for 15 minutes at 12,000 rpm at 4 ° C after standing at room temperature for 5 minutes.

Step 3.
Centrifuge the supernatant and transfer to a new RNase free EP tube. Add an equal volume of isopropanol and gently mix thoroughly. Reverse 6-8 times and let stand at room temperature for 10 minutes.

4.Centrifuge at 12,000 rpm for 10 min at 4 ° C and collect RNA pellet.

Step 4.
Wash twice with 75% ethanol and centrifuge at 12,000 rpm for 10 min.

Step 5.
Add an appropriate amount of DEPC water to dissolve the precipitate.For long-term preservation, then placed at -80 ℃.

Step 6.
Determine the concentration of RNA by measuring the absorbance at 260 nm.

Step 7.

8.Prepare the reverse transcription reaction system (20 µl) as follows to synthesize cDNA(Reverse Transcription cDNA kit (TransGen Biotech, Beijing, China))

①The first step(65℃ 5min, then immediate cooling on the ice 2min)

Total RNA 50ng-5ug

Oligo□dT□18 Primer□0.5ug/ul□ 0.5ul

Random Primer（0.1ug/ul） 0.5ul

②The second step(25℃ 10 min, 42℃ 15 min, 85℃ 5 seconds.)

2×TS Reaction Mix 10ul

RT/RI Enzyme Mix 1ul

gDNA Remover 1ul

RNase-free Water add up to 20 μl

Step 8.

Prepare the following qPCR system (20 μl) on ice(SYBR® Select Master Mix (Life Technologies, USA))

cDNA mixture 1 μl

SYBR® Select Master Mix（2x） 10ul

upstream primer (10 mM) 1 μl

downstream primer (10 mM) 1 μl

RNase free water add up to 20 μl

10.Set up the qPCR reaction procedure as described below:

Step 1 50℃ 2min

Step 2 95℃ 2 minutes

95℃ 15 seconds

60℃ 1 minutes

60℃ 10 seconds

40 Cycles in Step 2

Step 3 72℃ 5 minutes

Step 4 4℃ hold

Step 9.

Calculate relative gene expression by comparison of the CT value of the gene of interest with that of GAPDH which ia an internal control.