Quantitative Real-Time RT-PCR Assay Applying Calibrated mRNA Reference (Ctrl Mix)

Liu ZL

Abstract

Citation: Liu ZL Quantitative Real-Time RT-PCR Assay Applying Calibrated mRNA Reference (Ctrl Mix). **protocols.io** dx.doi.org/10.17504/protocols.io.nradd2e
Published: 08 Mar 2018

Protocol

Step 1.
Quantitative Real-Time RT-PCR Assay Applying Calibrated mRNA Reference (Ctrl Mix)

Step 2.
I. Reverse transcription reaction

Step 3.
1. For each set of labeling reaction, add the following and mix gently:

Step 4.
1) Purified total RNA (2 μg) ¬_ μl

Step 5.
2) Oligo (dT)18 (0.5 μg/μl) 1 μl

Step 6.
3) dNTP Mix (10mM) 1 μl

Step 7.
4) Ctrl Mix 1 μl

Step 8.
5) H2O ¬¬_ μl

Step 9.

Step 10.
Total 13 μl

Step 11.
2. Mix well and incubate at 65°C for 5 min

Step 12.
3. Chill on ice for at least 1 min

Step 13.
4. Add the following and mix gently:

Step 14.
1) 5x First Strand Buffer 4 μl

Step 15.

2) 0.1 M DTT 1 µl

Step 16.
3) SuperScript III (200 U/µl) 1 µl

Step 17.
4) RNaseOUT (40 U/µl) 1 µl

Step 18.

_____

Step 19.
Total 7 µl

Step 20.
Final reaction volume = 20 µl

Step 21.
5. Incubate at 50°C for 1 hr, 70°C for 15 min, and 4°C to end the reaction using a PCR cycler

Step 22.
II. PCR Reaction

Step 23.
6. For each reaction, add the following and mix well gently:

Step 24.
1) 2x SybrGreen MasterMix ¬12.5 µl

Step 25.
2) Forward Primer (10 mM) 0.5 µl

Step 26.
3) Reverse Primer (10 mM) 0.5 µl

Step 27.
4) Template 0.25 µl

Step 28.
5) H2O ¬¬ 11.25 µl

Step 29.

_____

Step 30.
Total 25 µl

Step 31.
7. Run reactions with the following thermal profile:

Step 32.
Stage 1: 95°C for 3 min

Step 33.
Stage 2: 40 cycles of

Step 34.
[Step 1: 95°C for 15 sec and

Step 35.
Step 2: 60°C for 45 sec]

Step 36.
Stage 3: Step 1: 95°C for 15 sec

Step 37.
Step 2: 60°C for 1 min and

Step 38.
Step 3: 95°C for 15 sec

Step 39.
Stage 4: Run dissociation curve

Step 40.
(Step 1: 95°C for 15 sec

Step 41.
Step 2: 60°C for 1 min and

Step 42.
Step 3: 95°C for 15 sec)

Step 43.
Settings:

Step 44.
Sample volume: 25 µl

Step 45.
Stat Collection: stage 2 step 2 (60°C @0.45)

Step 46.
Note: Run a separate dissociation program after completion of the PCR reaction in case it is not included in the instrument profile settings. This is to confirm the reaction is gene specific and free from the primer dimmer effects.