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Protocol status: Working
We use this protocol and it's working

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Quantitative real-time PCR V.1

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ABSTRACT

Quantitative real-time PCR protocol used for Day et al.

ATTACHMENTS

superscriptIV_VILO_master_mix_UG.pdf
Quantitative real.docx
TaqMan PreAmp Master Mix Protocol.pdf

MATERIALS

-SuperScript IV VILO Master Mix (Thermo Fisher Scientific)
-TaqMan PreAmp Master Mix (Thermo Fisher Scientific)
-TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific)
-UltraPure DNase/RNase-Free Distilled Water (Thermo Fisher Scientific)
-TaqMan Gene Expression Assays: hprt Mm03024075_m1, Slc12a2(NKCC1) Mm01265955_m1,Slc12a5(KCC2) Mm00803929_m1, Slc4a3 Mm00436654_g1, Slc4a10 Mm00473827_m1 (Thermo Fisher Scientific)
-PCR tubes, PCR plates and film
-Micropipettes
-Aerosol-resistant barrier pipette tips

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gDNA digestion

51m 15s

1 -For each sample prepare 10ul gDNA digestion reaction mix according to SuperScript IV VILO Master Mix protocol (see pdf attached or substeps below).

1.1 Digest gDNA for 00:02:00 min at 37 °C . 2m

1.2 Place the tubes on ice.

1.3 Add SuperScript IV VILO Master Mix and Nuclease-free water.

1.4 Gently mix and incubate at 25 °C for 00:10:00 . 10m

1.5 Then at 50 °C for 00:10:00 . 10m

1.6 Inactivate enzyme by incubation at 85 °C for 00:05:00 . 5m

Pre-amplification

51m 15s

2 Perform Pre-amplification according to TaqMan PreAmp Master Mix protocol (see pdf attached or substeps below).

2.1 -TaqMan PreAmp Master Mix 25ul, pooled assay mix (0.2X) 12.5ul, cDNA 2ul, nuclease free water, total 50ul.

2.2 Run reaction settings: 95 °C for 00:10:00 then 95 °C for 00:00:15 , 60 °C for 00:04:00 (10 cycles), inactivate enzyme 99 °C for 00:10:00 , hold at 4 °C 24m 15s

3 -Dilute each reaction 10 times.

Amplification

2m 41s

4 -PCR reaction mix: Gene Expression Assay (20X) 1ul, Preamplified cDNA product 5ul, TaqMan Fast Advanced Master Mix (2X) 10ul, nuclease free water 4ul, total 20ul.

5 -Run the reactions: Using incubation 50 °C for 00:02:00 then enzyme activation- 95 ° 2m 41s

00:00:20 , Denature 95 °C for 00:00:01 , Anneal/Extend 60 °C for 00:00:20 40 cycles.

Analysis

6 Experimental Ct values were normalized to hprt values using the following formula: ΔCt = Ct (gene of interest) − Ct (hprt). The final expression levels were shown as ΔCt values.