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Version 1

Quantitative real-time PCR V.1

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Protocol status: Working

We use this protocol and it's working

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Abstract

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

Attachments



[superscriptIV_VILO_m...](#)

1.2MB



[Quantitative real.do...](#)

15KB



[TaqMan PreAmp](#)

[Master...](#)

397KB

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: hpvt 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



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

2m 41s

Analysis

- 6 Experimental Ct values were normalized to hprt values using the following formula: $\Delta Ct = Ct \text{ (gene of interest)} - Ct \text{ (hprt)}$. The final expression levels were shown as ΔCt values.