



VERSION 1
DEC 18, 2023

OPEN ACCESS



DOI:
dx.doi.org/10.17504/protocols.io.e6nvwd4o2lmk/v1

Protocol Citation: Tatiana Tkatch 2023. Quantitative real-time PCR. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.e6nvwd4o2lmk/v1>
Version created by [c.weber-schmidt](#)

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

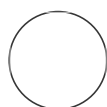
Protocol status: Working
We use this protocol and it's working

Created: Dec 18, 2023

Quantitative real-time PCR V.1

Tatiana Tkatch¹

¹Northwestern University



c.weber-schmidt
Allen Institute, ASAPCRN

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

Last Modified: Dec 18, 2023

PROTOCOL integer ID:
92468

Keywords: qPCR, real time
PCR

Funders

Acknowledgement:

Aligning Science Across
Parkinson's (ASAP)
Grant ID: ASAP-020551

NIH
Grant ID: NS 34696

JPB Foundation

CHDI Foundation

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

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: $\Delta Ct = Ct \text{ (gene of interest)} - Ct \text{ (hprt)}$. The final expression levels were shown as ΔCt values.