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Protocol — Quantitative Real-Time PCR (qRT-PCR): 3K-SNCA cells

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We use this protocol and it's working

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Abstract

Gene expression analysis in differentiated and undifferentiated 3K-SNCA SH-SY5Y cells using Qiagen RNA extraction and reverse transcription, followed by TaqMan® qRT-PCR on the QuantStudio® 1 platform.

Materials

- RNeasy Mini Kit (Qiagen, Cat# 74134)
- QuantiTect® Reverse Transcription Kit (Qiagen, Cat# 205311)
- TaqMan® Gene Expression Master Mix (Applied Biosystems, Cat# 436016)
- TaqMan® Gene Expression Assay — HEXA (Applied Biosystems, Cat# 4448892)
- TaqMan® Gene Expression Assay — GLA (Applied Biosystems, Cat# 4453320)
- TaqMan® Gene Expression Assay — GAPDH (Applied Biosystems, Cat# 4331182)
- QuantStudio® 1 Real-Time PCR System (Applied Biosystems)
- 6-well plates; RNase-free tubes and tips; plate seals
- DNase/RNase-free water; 70% ethanol
- Optional: gDNA wipeout/DNase treatment (per lab SOP)

Safety warnings

Follow RNA handling best practices (RNase-free technique, PPE). Ethanol is flammable; handle away from ignition sources.

Before start

- Warm reagents per manufacturer. Clean area with RNase decontaminant. Pre-label wells/plates.

Procedure — RNA extraction (Qiagen RNeasy Mini Kit)

1 Lyse cells directly in the 6-well plate (add appropriate volume of RLT buffer with β-mercaptoethanol).

2 Homogenise lysate and proceed with binding to RNeasy spin column per kit instructions.

3 Wash with RW1 and RPE buffers; dry spin to remove ethanol.

4 Elute RNA in RNase-free water (e.g., 30–50 µL). Measure concentration and purity (A260/280).

Procedure — cDNA synthesis (QuantiTect® RT Kit)

5 Prepare RT master mix on ice according to kit protocol.

6 Combine RNA (typically 500 ng–1 µg) with RT master mix to desired reaction volume.

7 Incubate per kit cycling (genomic DNA wipeout if applicable) and hold on ice.

Procedure — qPCR setup (TaqMan®)

8 Prepare TaqMan® master mix for each assay (Master Mix + 20× Assay + nuclease-free water).

9 Dispense master mix into wells and add cDNA template (equal volume across wells).

10 Seal plate, briefly spin to remove bubbles.

11 Hold: 95 °C for 10 min

12 PCR (40 cycles): 95 °C for 15 s; 60 °C for 60 s (data acquisition at anneal/extend step)

Data analysis — ΔΔCt method

13 Confirm amplification plots and absence of signal in NTC/−RT controls.

14 For each sample and target, compute ΔCt = Ct(target) − Ct(GAPDH).

15 Select a calibrator group (e.g., undifferentiated control). Compute ΔΔCt = ΔCt(sample) − mean ΔCt(calibrator).

16 Fold-change = 2^(−ΔΔCt). Report mean ± SEM from biological replicates.

Expected results

17 Reproducible triplicate Ct values (SD ≤ 0.3 cycles). Clear separation between target and NTC/−RT.

Troubleshooting

18 High Ct variability → check pipetting accuracy and RNA integrity.

19 Signal in NTC/−RT → assess contamination or gDNA carryover; include DNase step.

20 Low efficiency → verify assay IDs and storage; avoid freeze-thaw cycles.

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- Prepare TaqMan® master mix for each assay (Master Mix + 20× Assay + nuclease-free water).
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- Seal plate, briefly spin to remove bubbles.

Suggested cycling (typical for TaqMan® chemistry — adjust per kit)

- Hold: 95 °C for 10 min
- PCR (40 cycles): 95 °C for 15 s; 60 °C for 60 s (data acquisition at anneal/extend step)

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