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**ABSTRACT**

RT-PCR is a technique to quantify gene expression in samples

**PROTOCOL**

Gene expression was performed on tissue samples of the ventral midbrain (containing the substantia nigra pars compacta, SNpc).

***RNA Extraction***

At due time points, tissue samples were homogenized in 1ml of QIAzol Lysis Reagent (Qiagen, #79306) using a rotor-stator homogenizer. Total RNA was isolated from homogenized tissue samples using RNeasy Lipid Tissue Kit (Qiagen, #74804) including Dnase digestion. At the end, RNA samples were redissolved in 30 μl of RNase-free water and their concentrations were determinated spectrophotometrically by A260 (Nanodrop-ND 1000).

***Reverse Transcription***

cDNA synthesis were performed using Retroscript Kit (Ambion #1710, Austin, Texas) according the manufacturer’s instructions. 50 μl of water solution containing 0.5 μg of each pool were added to an equal volume of 2X TaqMan Universal PCR Mastrer Mix (Applied Biosystems).

***Real Time PCR***

Real-time quantitative PCR was performed using TaqmanTMAssay Reagents on an Step One Detection System (Applied Biosystems) according to manufactures protocol. Tissue samples were processed as above. Cell samples were lysed in lysis buffer (Qiagen) and stored at -80°C until the RNA was extracted following manufacture’s instructions. Residual genomic DNA was removed by incubating with DNase I, RNase-free (Qiagen) and eluted from the RNeasy mini columns with RNase-free water. The amount of total RNA was quantified using a NanoDrop ND-100 (Nano Drop Technologies) and the cDNA was synthesized from 2 μg of total RNA using the Retroscript Kit (Ambion). After purification using QIAquick PCR Purification kit (Qiagen), 250 ng of cDNA were used for Real-time PCR using pre-developed Taqman Assay Reagents (Applied Biosystems). Real-time quantitative PCR was performed with Step One Detection System (Applied Biosystems) according to manufacturer protocol. We used the housekeeping gene, β-actin, as normalizer and embrionic mouse brain as calibrator. Quantification of the abundance of target gene expression was determined relative to β-actin with respect to the control group by using the delta delta Ct (2-ΔΔCt) comparative method, the results expressed as arbitrary units (AU). Relative fold changes over WT in the respective treatment groups are indicated.