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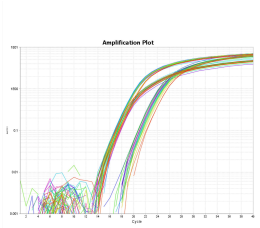
iNeuron RNA-Extraction and RT-qPCR



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We use this protocol and it's working

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Abstract

Protocol for the extraction of RNA from iNeurons and RT-qPCR measurements of *PINK1* mRNA.

Materials

- Monarch Total RNA Miniprep Kit (New England Biolabs, T2010S)
- UltraPure DNase/RNase-Free Distilled Water (Invitrogen, 10977035)
- SuperScript IV Reverse Transcriptase (Invitrogen, 18090050)
- dNTP Mix (10 mM each) (Invitrogen, R0192)
- Random Hexamers (50µM((Invitrogen, N8080127)
- RNaseOUT Recombinant Ribonuclease Inhibitor (Invitrogen, 10777019)
- Power SYBR Green PCR Master Mix (Applied Biosystems, 4367659)
- Gene Specific Primers (Sigma)
- NanoDrop One Spectrophotometer (Thermo Fisher Scientific)
- QuantStudio 7 Flex Real-Time PCR System (Applied Biosystems, RRID:SCR_020245)

RNA Extraction

1 iNeuron culture and initial collection for RNA extraction described in "iNeuron Differentiation and Culture in N2B27 vs BrainPhys for Immunofluorescence and Biochemistry Assessments of Mitophagy" (https://www.protocols.io/private/E5851436F8F111EF86990A58A9FEAC02).
RNA is extracted using the Monarch Total RNA Miniprep Kit (New England Bioscience) as per the manufacturers instructions and with inclusion of gDNA binding column and the optional on-column DNAse treatment.
RNA is eluted from the column in 30 µL of Nuclease free H2O

1.1 RNA is then quantified by 260nm absorbance using a NanoDrop One Spectrophotometer.

Reverse Transcription

2 100ng of RNA is reverse transcribed in a 10 µL reaction with [M] 2.5 U/µl SuperScript IV reverse transcriptase, [M] 2.5 micromolar (µM) random hexamers, [M] 0.5 millimolar (mM) dNTPs, [M] 5 millimolar (mM) DTT and [M] 2 U/µl RNAseOUT.
A 100ng reverse transcription negative control reaction consisting of the same reaction mixture above but without reverse transcriptase is also conducted for each sample.
The reverse transcription and negative control reactions are thermocycled at:

	Temperature	Time	Cycles
	25°C	10min	x1
	50°C	40min	
	80°C	10min	

Reverse Transcription Thermocycle

2.1 The cDNA product and reverse transcription negative control reactions are then diluted such that 100 ng of reverse transcribed RNA would be in a 120 µL final volume (i.e. add 10 µL reaction to 110 µL Nuclease Free H2O = [M] 0.83 ng/µl).

RT-qPCR

3 3.33ng of cDNA (4 µL of the [M] 0.83 ng/µl dilution) is used in each well of a 10 µL RT-qPCR reaction using Power Sybr Green PCR master mix, [M] 500 nanomolar (nM) gene-specific primer pairs and the QuantStudio 7 Flex Real-Time PCR System.
Each sample and gene target reaction is conducted with at least 2x technical replicates, with the same volume of reverse transcription negative control conducted alongside in a separate well for each gene target sample pair.

RT-qPCR Primer Pairs
PINK1_F: 5′- GTGGAACATCTCGGCAGGTT-3′
PINK1_R: 5′- CCTCTCTTGGATTTTCTGTAAGTGAC-3′
UBC_F: 5′-CTGGAAGATGGTCGTACCCTG-3′
UBC_R: 5′- GGTCTTGCCAGTGAGTGTCT-3′.

3.1 Ct values are determined automatically using the Quantstudio Real-time PCR Software and relative *PINK1* mRNA expression levels are calculated using the $2^{-\Delta\Delta Ct}$ method with *UBC* as the house-keeping gene.

Protocol references

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