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RNA collection, cDNA conversion and qPCR (SH-SY5Y cells)

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We use this protocol and it's working

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Abstract

This protocol describes the isolation of RNA from SH-SY5Y cells and the subsequent conversion to cDNA for qPCR.

Materials

- NucleoSpin RNA plus kit: 740984.50, Macherey-Nagel
- High-Capacity cDNA Revers Transcription Kit: 4368814, Thermo Fisher Scientific
- SYBR Green master mix: 04707516001, Roche

RNA collection

1 Cells were seeded in 10 cm dishes and used for collection when reaching 70-80% confluency. (*e.g.* 3 million cells for collection after 48:00:00) 2d

2 Remove medium.

3 Wash once with PBS (-/-).

4 Scrape and collect cells in PBS(-/-).

5 Spin down cells (450xg, 00:05:00). 5m

6 Wash with PBS(-/-).

7 Spin down cells (450xg, 00:05:00). 5m

8 Remove supernatant.

9 Isolate RNA following the instructions of the NucleoSpin RNA Plus kit (740984.50, Macherey-Nagel).
! Use a separate, desinfected area to isolate RNA. Use filter tips and dedicated pipets for RNA work.

10 Determine the concentration and the purity of the isolated RNA using a Nanodrop spectrometer.

cDNA conversion

11 Convert RNA to cDNA using the High-Capacity cDNA Reverse Transcription Kit (4368814, Thermo Fisher Scientific).

11.1 Prepare 5 µg RNA in 20 µl total volume (dilute with RNase free water).

11.2 Prepare a 2x Mastermix:

	A	B
	Volume (µl)	Component
	2	µl RT buffer
	0,8	µl dNTPs (100 µM)
	2	µl random primers (10x)
	1	µl Multiscribe transcriptase
	4,2	µl AD

Volumes are given for one sample, multiply according to your number of samples.

11.3 Add 10 µl of the mastermix to 10 µl of the RNA dilution.

11.4 Perform a quick vortex and spin down using a table-top centrifuge.

11.5 Start program for RNA to cDNA conversion:

	A	B	C	D	E
		Step 1	Step 2	Step 3	Step 4
	Temp (°C)	25	37	85	4
	Time	10 min	120 min	5 sec	∞

qPCR

12 Prepare a serial dilution of the sample that you choose as standard (1/5, 1/25, 1/125, 1/625, 1/3125). Include water as a negative control.
Pipet in duplo in a 96-well plate (5 µl per well).

13 Prepare a master mix containing per sample:
- 10 µl SYBR Green master mix (Roche)
- 1 µl of 5 µM forward primer
- 1 µl of 5 µM reverse primer
- 3 µl water

14 Prepare a ten-fold dilution of the cDNA samples in duplicates (5 µl cDNA per well).
Include a negative control where the cDNA is exchanged by an equivalent volume of water.

15 Add 15 µl of the master mix to each well.

16 Cover plate with a film and spin samples down.

17 Start the qPCR reaction: 11m 40s
- 95 °C for 00:10:00
- 50 cycles at 95 °C for 00:00:10
- 55 °C for 00:00:30
- 95 °C for 00:01:00

Determine a melting curve from 55 to 95 °C.