Nanovesicles extraction

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ABSTRACT

Nanovesicles extraction (Exosomal isolation, SHSY-5Y)

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1 seed cells for **24:00:00** in 3 x 15 cm dishes at a density of 20 x 10^6 cells per plate 1d

2 wash cells of all the plates with 2 times **10 mL** of 1x PBS

3 Add **10 mL** DMEM with 1% exosome depleted FBS to each plate for **24:00:00** 1d

Preparation of exosome depleted FBS:
Commercial FBS was filtrated through 0.22 µm filter and **140000 x g , overnight**

4 collect medium.

purify nanovesicles with differential centrifugation (see substeps)

4.1 **300 x g, 00:15:00** (remove cells) 15m

4.2 **15000 x g, 00:30:00** (remove cell debris) 30m

4.3 filter through 0.22 µm filter (filtration of apoptotic bodies)

4.4 **140000 x g, 4°C, 03:00:00** 3h

5 decant supernatant and collect nanovesicles in **50 µl** cell lysis buffer (for western blotting) or 50 µl PBS+ PI (for intact cell cross-linking)

Intact cell cross-linking
by Joris Van Asselberghs
PREVIEW RUN

5.1 seed cells (SHSY-5Y) in 10 cm dishes at a density of 6 x 10^6 cells per plate. 1d
incubate for **24:00:00**

5.2 wash cells 2 times with 1X PBS

5.3 harvest cells with 1x PBS + PI cocktail (PBS + PI buffer)

5.4 **1000 x g, 00:15:00** and resuspend in **400 µl** 15m
1X PBS (Phosphate-buffered saline) **Contributed by users** + PI buffer

5.5 divide **400 µl** resuspended cells over 2 vials 30m
tube 1: add 1 µM disuccinimidyl glutarate (DSG; Thermo Fisher Scientific)
tube 2: add 1µM dithiobis succinimidyl propionate (DSP; Thermo Fisher Scientific

incubate **00:30:00** at **37 °C**

Preparation of 50X DSP, 50X DSG

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50X DSP: 2.02mg into 100µL DMSO
50X DSG: 1.632mg into 100µL DMSO

Note: Prepare the crosslinkers freshly before use

5.6 quench reaction for **00:15:00** on **Room temperature** in both tubes with **20 Milimolar (mM)** Tris (pH **7.4**) 15m

5.7 sonicate cells 2 times at 30 Hz with 15 ON-OF intervals of **00:00:10** (1s pulses) 10s

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