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Intact cell cross-linking

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1 Works for me

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ABSTRACT

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PARENT PROTOCOLS

In steps of

[Nanovesicles extraction](#)

- 1 seed cells (SHSY-5Y) in 10 cm dishes at a density of 6×10^6 cells per plate. incubate for **24:00:00** 1d
- 2 wash cells 2 times with 1X PBS
- 3 harvest cells with 1x PBS + PI cocktail (PBS + PI buffer)
- 4 **1000 x g, 00:15:00** and resuspend in **400 μ l** 15m

☒ 1X PBS (Phosphate-buffered saline) Contributed by users + PI buffer

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





incubate  00:30:00 at  37 °C

Preparation of 50X DSP, 50X DSG

50X DSP: 2.02mg into 100µL DMSO

50X DSG: 1.632mg into 100µL DMSO

Note: Prepare the crosslinkers freshly before use

- 6 quench reaction for  00:15:00 on  Room temperature in both tubes with  20 Millimolar (mM) Tris (^{15m}
 7.4)

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