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Synaptosome extraction

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We use this protocol and it's working

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Abstract

Synaptosome extraction

Troubleshooting

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- 1 Synaptosomes (SYNs) are then extracted from mature iPSC-derived cortical neurons using Syn-PER Synaptic Protein Extraction Reagent (Thermo Fisher Scientific).
- 2 1x of protease inhibitors (Roche) are added to the Syn-PER Reagent immediately before use. 200 μ L of Syn-PER Reagent was added to each 6-wp well and cells were lifted from the surface using a cell scraper (Biologix).
- 3 Samples are centrifuged at 1200xg for 10 minutes at 4°C.
- 4 Supernatant is transferred to a new tube and centrifuged at 15000xg for 20 minutes at 4°C. Pellet containing SYNs was resuspended in Syn-PER Reagent.
- 5 SYNs concentration is measured using the Bradford assay solution (Bio-Rad Laboratories)
- 6 Protein concentration is quantified by optical density in a PowerWave XS multiplate reader (Biotek).
- 7 Resulting SYNs are frozen in 5% (v/v) Dimethyl sulfoxide (DMSO) at -80 °C for extended periods, until pHRodoTM Red (ThermoFisher Scientific) conjugation.