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Gcase co-immunoprecipitation

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michela.deleidi[1], Federico Bertoli[1], Maria Jose Perez J.[1], Hariam Raji[1]

[1]German Center for Neurodegenerative Diseases (DZNE), Tübingen, 72076 Germany

Federico Bertoli
ASAP

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Protocol status: Working
We use this protocol and it's working

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Abstract

We developed this protocol to identify protein-protein interactions between the enzyme glucocerebrosidase (GCase) and other proteins in human iPSC-derived Neural Precursor Cells.

Attachments

DOCX

676- 1426.docx

13KB

Materials

Pierce™ IP Lysis Buffer **Thermo Fisher** **Catalog #**87787

Pierce Protease and Phosphatase Inhibitor Mini Tablets **Thermo Fisher** **Catalog #**A32959

Pierce™ Crosslink Magnetic IP/Co-IP Kit **Thermo Fisher** **Catalog #**88805

Pierce Protein A/G Magnetic Beads **Thermo Fisher Scientific** **Catalog #**88802

Gcase co-immunoprecipitation

2h 5m

1 Wash cells 1X with phosphate-buffered saline (PBS, Sigma–Aldrich) and detach using Accutase.

2 Pellet the cell suspension at 280 rcf, 23°C, 00:05:00 . 5m

3 Lyse the pellets in IP/lysis buffer (Thermo Fisher, #87787) supplemented with a protease/phosphatase inhibitor cocktail (Pierce, #A32959).

4 Carry out coimmunoprecipitation using the Thermo Fisher Pierce Crosslink Magnetic IP/Co-IP Kit (#88805) according to the manufacturer´s instructions:

5 Prewash 25 µL of Pierce protein A/G magnetic beads (Thermo Fisher, #88802-3) twice with 1X Modified Coupling Buffer and incubate with 10 µg of GBA MaxPab polyclonal rabbit antibody (Abnova) or normal rabbit IgG (Covalab, #pab01004-P) on a rotating wheel Overnight at 4 °C .

6 The following day, crosslink the antibody to the beads with a [M] 0.25 millimolar (mM) DSS solution for 01:00:00 on a rotating wheel at Room temperature . 1h

7 Incubate crosslinked magnetic beads Overnight with a total of 7 mg of protein for each lysate. 1h

8 Elute Coimmunoprecipitated proteins from the beads with 60 µL of the kit-provided Elution buffer pH 2.0 (Pierce, #88805) and neutralize with 6 µL of Neutralization Buffer provided with the kit.

9 Prepare samples for Western blotting by adding 5x Lane buffer +10% DTT [M] 1 Mass Percent to a final concentration of 1X.

Note

- Each western blot input sample loaded corresponds to a total of 50 µg of protein.
- Each western blot CoIP sample loaded corresponded to the total of each elution product (66 µL).