



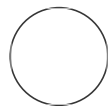
MAR 30, 2023

Gcase co-immunoprecipitation

In 1 collection

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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

[676-1426.docx](#)

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**

OPEN ACCESS

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

Created: Mar 30, 2023















Last Modified: Mar 30, 2023

PROTOCOL integer ID:
79780

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  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  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  1 Molarity (M) 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).