Mar 31, 2023

Gcase co-immunoprecipitation

In 2 collections

DOI
https://dx.doi.org/10.17504/protocols.io.kxygx9xozg8j/v1

michela.deleidi[1], Federico Bertoli[1]

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

Federico Bertoli
ASAP

DOI: https://dx.doi.org/10.17504/protocols.io.kxygx9xozg8j/v1

Protocol Citation: michela.deleidi , Federico Bertoli 2023. Gcase co-immunoprecipitation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygx9xozg8j/v1

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

Created: March 31, 2023

Last Modified: May 31, 2024

Protocol Integer ID: 79780

Keywords: ASAPCRN, protein interactions between the enzyme glucocerebrosidase, enzyme glucocerebrosidase, glucocerebrosidase, other proteins in human ipsc, neural precursor cell, protein, derived neural precursor cell, gcase, other protein, protein interaction, immunoprecipitation, enzyme, human ipsc

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).