**GCase coimmunoprecipitation**

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**ABSTRACT**

GCase coimmunoprecipitation (Co-IP) is a technique used to identify protein-protein interactions between the enzyme glucocerebrosidase (GCase) and other proteins. We applied this in human iPSC derived Neural Precursor Cells.

**Protocol**

Cells were washed 1X with phosphate-buffered saline (PBS, Sigma‒Aldrich) and detached using Accutase. The cell suspension was pelleted at 280 rcf for 5’ at 23°C.

The pellets were then lysed in IP/lysis buffer (Thermo Fisher, #87787) supplemented with a protease/phosphatase inhibitor cocktail (Pierce, #A32959). Coimmunoprecipitation was carried out using the Thermo Fisher Pierce Crosslink Magnetic IP/Co-IP Kit (#88805) according to the manufacturer´s instructions. Twenty-five microliters of Pierce protein A/G magnetic beads (Thermo Fisher, #88802-3) were prewashed twice with 1X Modified Coupling Buffer and incubated 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. The following day, the antibody was crosslinked to the beads with a 0.25 mM DSS solution for 1 h on a rotating wheel at RT. Crosslinked magnetic beads were incubated overnight with a total of 7 mg of protein for each lysate. Coimmunoprecipitated proteins were eluted from the beads with 60 µl of the kit-provided Elution buffer pH 2.0 (Pierce, #88805) and neutralized with 6 µl of Neutralization Buffer provided with the kit. Samples were prepared for Western blotting by adding 5x Lane buffer +10% DTT 1 M to a final concentration of 1X. 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).