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Co-Immunoprecipitation (co-IP) Protocol

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

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Abstract

Co-immunoprecipitation (co-IP) is a widely utilized technique for studying protein-protein interactions, particularly in cellular models.

Materials

- 1x PBS (Phosphate-Buffered Saline)
- 1:10 diluted extraction buffer:
- 50 mM HEPES, pH 7.4
- 40 mM NaCl
- 2 mM EDTA
- 1% Triton X-100
- 1.5 mM sodium orthovanadate (NaVO4)
- 30 mM sodium fluoride (NaF)
- 10 mM sodium pyrophosphate (Na4P2O7)
- 10 mM sodium β-glycerophosphate
- Protease inhibitor cocktail
- Anti-Flag magnetic beads (Pierce™ Anti-DYKDDDDK Magnetic Agarose (Anti-FLAG)(Thermo Fisher Scientific)
- SDS-PAGE materials (Thermo Fisher Scientific)
- Loading dye (5x and 1x)
- Centrifuge
- Shaker
- Incubator at 4°C
- Immunoblotting equipment

Extraction Buffer (1x):

- **50 mM HEPES**, pH 7.4
- **40 mM NaCl**
- **2 mM EDTA**
- **1% Triton X-100**
- **1.5 mM Sodium Orthovanadate (NaVO4)**
- **30 mM Sodium Fluoride (NaF)**
- **10 mM Sodium Pyrophosphate (Na4P2O7)**
- **10 mM Sodium β-Glycerophosphate**

Cell Culture

3d

1 Seed 15 × 10^6 SH-SY5Y cells expressing the Flag-fusion protein (here: Flag-tagged SLC45A1) and control cells in 15 cm dishes

1.1 Incubate the cells for 72:00:00 at 37 °C in a humidified atmosphere with 5% CO_2, or until the cells reach confluence

3d

Cell Lysis

1h

2 Wash the cells gently with 10 mL 1x PBS to remove any residual media.

2.1 Prepare 1 mL of **1:10 diluted extraction buffer** supplemented with a protease inhibitor cocktail.
Extraction buffer: Please see "Materials"

2.2 Add the extraction buffer and lyse the cells. Use a cell lifter to collect the cells, then transfer the lysate to a new tube.

It is highly recommended to use low-protein-binding tubes for all steps.

2.3 Incubate lysate at 4 °C with vortexing for 01:00:00

1h

2.4 Centrifuge the lysate at maximum speed for 00:10:00 at 4 °C

10m

2.5 Carefully collect the supernatant to new tube

2.6 Take 100 µL of the supernatant and add loading dye. Mix well and set aside for later use as the input sample.

Immunoprecipitation

1h

3 Transfer the remaining 900 µL of supernatant to a new tube containing 25 µL of **pre-washed Anti-Flag magnetic beads** (Beaded were washed with **1:10 diluted extraction buffer**).

3.1 Incubate the mixture at 4 °C with gentle rotation overnight. Alternatively, a shorter incubation of 3 hours can also be used if desired

3.2 After incubation, wash the beads **seven times** with **1 ml** of **1:10 diluted extraction buffer**. Use a magnet to facilitate the separation of the magnetic beads during the washing process

Important: Change tubes every two washes to ensure effective washing.

3.3 After the final wash, add 50 µL of 1x loading dye to the beads. Mix thoroughly.

3.4 Alternatively, if non-denatured protein samples are needed, incubate the beads with 200 µg/mL Flag-peptide (prepared in 1:10 diluted extraction buffer 01:00:00 to 02:00:00 at 4 °C 3h

It is recommended to optimize the Flag-peptide concentration and incubation time for your specific Flag-fusion protein