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Rapid Isolation of Lysosomes (LysoIP) from mouse brain tissue

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We use this protocol and it's working

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Abstract

This protocol outlines a method for the rapid isolation of lysosomes from fresh mouse brain tissue. Lysosomes serve as metabolic hubs for the degradation and recycling of biomolecules. Despite their critical cellular functions, quantitatively assessing lysosomal protein profiles has been challenging. To address this, we developed a rapid harvesting and purification method using immunoprecipitation (LysoIP). This protocol provides detailed instructions for preparing LysoIP from mouse brain tissue for proteomic analysis.

Materials

1x extraction buffer :

- 50 mM HEPES, pH 7.4
- 40 mM NaCl
- 2 mM EDTA
- 1% Triton X-100 (Sigma)
- 1.5 mM sodium orthovanadate (NaVO4)
- 30 mM sodium fluoride (NaF)
- 10 mM sodium pyrophosphate (Na4P2O7)
- 10 mM sodium β-glycerophosphate
- Complete EDTA-free Protease Inhibitor Cocktail

Materials

1 Materials and Reagents

1. **Anti-HA magnetic beads:** Thermo Fisher Scientific
2. **Optima LC/MS water**
3. **Phosphate-buffered saline (PBS):** pH ~7.4
4. **Complete EDTA-free Protease Inhibitor Cocktail:** Roche
5. **Magnetic separation equipment:** DynaMag Spin Magnet
6. **1x extraction buffer (Material section)**
7. **Centrifuge**
8. **Homogenizer:** 2 ml homogenizer

Equipment Preparation

2 Wash the glass vessel homogenizer with MilliQ Water, 10 times each. wash the tissue grinder
homogenizer thoroughly with DI Water and MilliQ Water, especially the gap between the white
parts, don't touch the part that goes into the glass vessel. Then dry upside-down using paper
towels. Carefully place the glass vessels against something to prevent falling down. Minimize
any contact between the grinder and anything else.

- **Pre-chill all equipment and buffers** used for homogenization and immunoprecipitation to 4°C.
- **Set centrifuge temperature to 4°C** for all centrifugation steps.

3 **Prepare microcentrifuge tubes as follows on a metal rack on ice (for each sample, from left to
right):**
① 2 mL tube for posthomogenization
② 1.5 mL tube for whole brain fraction
③ 1.5 mL tube for beads
④ 1.5 ml tube for 3rd wash
⑤ 1.5 mL tube for final lysoIP samples

Preparation of Anti-HA beads

4 Pool all required beads volumes together (100 µL / sample, e.g. 800 µL total for 8 samples, extra is not needed).

4.1 Wash 3 times with the same volume (100 µL / sample, e.g. 800 µL total for 8 samples) of ice-cold PBS containing Complete EDTA-free Protease Inhibitor Cocktail. After each wash, place the tube on the magnet, aspirate the supernatant, and add the same volume of ice-cold PBS containing Complete EDTA-free Protease Inhibitor Cocktail.

4.2 After thirs wash, Resuspend beads with the same volume (100 µL / sample, e.g. 800 µL total for 8 samples) of ice-cold PBS containing Complete EDTA-free Protease Inhibitor Cocktail.

4.3 Aliquot 100 µL into each ③ 1.5 mL tube for beads.

Isolation of Lysosomes from Mouse Brain

5 Tissue Collection and Preparation:

5.1 Process each mouse brain separately to ensure rapid isolation of lysosomes.

5.2 Euthanize the mouse and transfer the whole brain into 2 ml homogenizer (on ice)

Homogenization

6 **Critical: All steps of the procedure must be performed on ice**

6.1 Immediately add 950 µL ice-cold PBS containing Complete EDTA-free Protease Inhibitor Cocktail into 2 ml homogenizer.

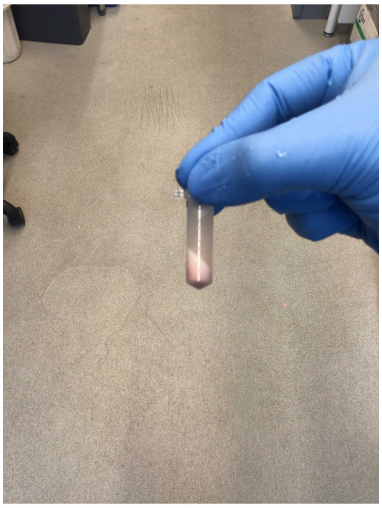
6.2 Homogenize with 25 gentle strokes (on ice).

6.3 Transfer the homogenized tissue to a 2 ml tube.

6.4 Reserve 25 µL of the homogenate (2.5% of the total tissue) for processing as the whole brain fraction.

6.5 **Recommended:** Add 1 ml of ice-cold PBS with Protease Inhibitor Cocktail to the 2ml tube (Step 6.3) to minimize pellet contamination after centrifuge (next step).

6.6 Centrifuge at 1000 x g, 00:02:00 at 4°C. 2m



Immunoprecipitation of Lysosomes 15m 25s

7 Transfer the supernatant containing cellular organelles to a 1.5 ml tube containing 100 µL of anti-HA magnetic beads

7.1 Incubate on a gentle rotator shaker for 00:15:00 at 4 °C 15m

7.2 Place the tube on magnet. Count at least 00:00:25 to allow for beads to be pulled by
magnets. 25s

Note: it is important to keep this count the same between each wash and each sample

for consistency I.e. 25 seconds each time .

7.3 Wash the beads fraction three times with 1 mL PBS containing Protease Inhibitor Cocktail using a DynaMag Spin Magnet.

Note:
- During the first wash, make sure to aspirate any liquid trapped on the inner side of the cap.
- Pipet up and down 3 times and keep consistent each wash, each sample.

7.4 After the second wash, resuspend beads with PBS containing Protease Inhibitor Cocktail and then transfer it to the clean ④ 1.5 ml tube for third wash (this step helps give cleaner results).

7.5 Place the tube on magnet wait 00:00:25 to allow beads to be pulled by magnets. 25s
Then, aspirate the supernatant.

Lysosomal Protein Extraction 30m

8 Resuspend the beads with 80 µL of ice-cold **1x extraction buffer.**

8.1 Incubate at least for 00:15:00 at 4 °C 15m

8.2 After 00:15:00 finishing the last IP, place the IP samples on the magnet to separate the beads. **Collect the supernatant and transfer it** to a new 1.5 ml tube. Store the IP samples at -80 °C until further processing. 15m

8.3 For whole brain fraction add 160 µL of ice-cold 1x extraction buffer. 25m

- Incubate at least for 00:15:00 at 4 °C
- Centrifuge 15000 rpm, 00:10:00 4 °C
- Transfer the supernatant to a new tube. Store the samples at -80 °C until further processing.

Quality Control

9

- Add loading dye to the sample before running it on SDS-PAGE. Load 1% of the whole brain lysate and 10% of LysoIP.

- Detect lysosomal markers (LAMP1, Cathepsin B), Golgi marker (Golgin-97), endoplasmic reticulum marker (Calreticulin), and mitochondrial marker (VDAC) to confirm the enrichment of lysosomes.