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Rapid Isolation of Lysosomes (LysoIP) from iPSC-Derived Neurons for Proteomics, with Options for Lipidomics/Metabolomics

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We use this protocol and it's working

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Abstract

This protocol describes rapid immunopurification of lysosomes (LysoIP) from cultured iNeurons expressing a lysosomal HA tag (e.g., TMEM192-3xHA). The workflow is optimized for proteome analysis from one 10-cm dish (~8–10×10^6 iNeurons) and can be scaled by pooling multiple dishes for lipidomics/metabolomics. The method relies on cold, rapid handling; gentle homogenization; anti-HA magnetic capture; stringent washes; and extraction compatible with downstream mass spectrometry.

Materials

Reagents

- **Anti-HA magnetic beads** (e.g., Thermo Fisher; same class as used in template)
- **PBS, pH 7.4** (ice-cold)
- **Complete EDTA-free Protease Inhibitor Cocktail** (Roche)
- **Optima LC/MS water** (if doing metabolite/lipid workflows)
- **KPBS** (for metabolite/lipid-compatible washes): 136 mM KCl, 10 mM KH2PO4, pH 7.25 (KOH; in MS-grade water)
- **Optima LC/MS methanol** (for metabolite extraction)
- (Optional) isotopically labeled internal standards for metabolomics

Buffers

A) Ice-cold PBS + Protease inhibitors (PBS+PI)

- PBS pH 7.4 + Complete EDTA-free PI (fresh)

B) 1× Extraction Buffer (for protein extraction)

- 50 mM HEPES pH 7.4
- 40 mM NaCl
- 2 mM EDTA
- 1% Triton X-100
- 1.5 mM sodium orthovanadate
- 30 mM sodium fluoride
- 10 mM sodium pyrophosphate
- 10 mM sodium β-glycerophosphate
- Complete EDTA-free PI

C) 80% MeOH (v/v), pre-chilled to freezing

- 80% methanol in LC/MS water (add internal standards if desired)(**For lipidomics or metabolomics)**

Equipment

- DynaMag (or equivalent) spin magnet
- Pre-chilled microcentrifuge (4 °C)
- Cell lifter/scraper
- 1.5 mL low-bind tubes (recommended)
- U-100 29G x 1/2 for homogenization
- Rotator (cold room or 4 °C)

Before you start (Critical)

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- Pre-chill all buffers, tubes, magnet, and centrifuge to 4 °C.
- Keep everything on ice and minimize time from harvest → beads.
- Label tubes for each sample (example structure used in the template: post-homogenization, whole-cell/whole-homogenate, beads, final IP).

Input guidance (iNeurons)

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- Proteomics (recommended starting point): 1 × 10-cm dish with ~8–10×10^6 differentiated cells per condition.

- Lipidomics/metabolomics: plan to pool multiple 10-cm dishes per condition (start with ~3 dishes; exact number depends on platform sensitivity and expected lysosome yield—optimize empirically).

Preparation of anti-HA beads

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1. **Pool beads**: use **100 µL beads per sample.**
2. **Wash beads 3X**

- For **proteomics**: wash 3× with **PBS+PI** (See Materials)
- For **metabolomics/lipidomics**: wash 3× with **cold KPBS** (MS-grade)

3. Resuspend beads to the original pooled volume and **aliquot 100 µL beads** into a labeled 1.5 mL tube for each sample.

iNeuron harvesting (10-cm dish)

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1. Place the plate on ice. Aspirate media.
2. Wash **2×** with ice-cold PBS (gentle).
3. Add **~950 µL** ice-cold PBS+PI (proteomics) **or** KPBS (metabolomics) to the dish.
4. Scrape with a cell lifter and transfer suspension to a pre-chilled **2 mL** tube.
5. Spin **1,000×g, 2 min, 4 °C**.
6. Aspirate supernatant and resuspend pellet in **950 µL** ice-cold PBS+PI (or KPBS for metabolomics).
7. Take **25 µL** as “whole cell/homogenate input” control.

Homogenization

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1. Homogenize the remaining suspension by passing through an **insulin syringe 2X** (keep on ice; avoid bubbles).

2. Clarify nuclei/unbroken cells: spin **1,000×g, 2 min, 4 °C**.

3. Carefully transfer the **supernatant (organelle-containing fraction)** to the tube containing **100 µL washed anti-HA beads**. Avoid disturbing the pellet.

Immunoprecipitation

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1. Incubate on a gentle rotator at **4 °C for 10 min** (proteomics workflow).

- (Metabolomics workflows often use a shorter timed **3 to 5 min** capture in cold conditions; maintain consistency across samples.)

2. Place on magnet and allow beads to collect (**keep timing consistent**, e.g., ~25 s).
3. Remove supernatant.

Washes (critical for purity)

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1. Wash beads **3×** with **1 mL** cold PBS+PI (proteomics) or **3× with 1 mL cold KPBS** (metabolomics/lipidomics).

- During first wash, remove any liquid trapped in the cap; pipet up/down consistently.

2. After the **second wash**, resuspend beads and **transfer to a fresh tube for the third wash** (clean-tube wash improves cleanliness).

3. After final wash, remove all residual buffer.

Elution / Extraction options

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Option A — Proteomics (protein extraction)

1. Add **80 µL** ice-cold **1× Extraction Buffer** to beads.

2. Incubate **15 min at 4 °C**.

3. Place on magnet, collect supernatant (protein extract), transfer to a new tube, store at **−80 °C**.

4. Process the **input control** by adding extraction buffer **165 µL** (template uses extraction buffer incubation then high-speed spin). Spin **top speed (~15,000 rpm), 10 min, 4 °C**, transfer supernatant, store −80 °C.

Option B — Polar metabolites (metabolomics)

1. Resuspend beads in **50 µL freezing-cold 80% MeOH** (include internal standards if used).
2. After final sample, magnet-separate and transfer the MeOH extract to a new tube; store **−80 °C**.
3. Extract whole-cell control similarly with 80% MeOH and clear by **top-speed spin (15,000 rpm, 10 min, 4 °C)**; store supernatant.

Option C — Non-polar metabolites (metabolomics)
Please follow the steps below for processing nonpolar lipid samples in this protocol:
Step: **Processing of nonpolar lipids samples**

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