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Small-scale LysoIP

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We use this protocol and it's working

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Abstract

This protocol describes a technique for native immunoprecipitation of intact lysosomes (LysoIP). There are many protocols for this technique, but this protocol has been adapted to be performed at a small scale and with an elution compatible for downstream immunoblot analysis, producing just enough material for several immunoblots.

Materials

- 10 cm plate of cells expressing TMEM192-3xHA
- 10 cm plate of an isogenic cell line lacking this tag (to control for nonspecific binding)
- TrypLE™ Express Enzyme (1X), phenol red **Thermo Fisher Catalog #**12605036
- 1X PBS PBS (10X), pH 7.4 **Thermo Fisher Scientific Catalog #**70011051
- DMEM, high glucose, pyruvate **Thermo Fisher Catalog #**11995073 with Fetal Bovine Serum **Gibco - Thermo Fisher Scientific Catalog #**10270106
- KPBS Buffer: 10 mM KH2PO4, 136 mM KCl, pH 7.25, 1X cOmplete™ EDTA-free Protease Inhibitor Cocktail **Roche Catalog #**11873580001
- RIPA Buffer: RIPA Lysis and Extraction Buffer **Thermo Fisher Catalog #**89900, 1X cOmplete, Mini EDTA-free Protease Inhibitor Cocktail
- NUPAGE LDS sample buffer (4x) **Thermo Fisher Scientific Catalog #**NP0007 + 10% 2-mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M6250
- Pierce™ Anti-HA Magnetic Beads **Thermo Fisher Catalog #**88836
- Dounce homogenizer vessel 2 mL (VWR cat. no. 89026-386)
- Dounce homogenizer plunger 2 mL (VWR cat. no. 89026-398)
- Magnetic Rack
- Centrifuge
- Tube rotator
- Nutator
- Pierce™ Rapid Gold BCA Protein Assay Kit **Thermo Fisher Catalog #**A53225

Bead Preparation

1 Add 75 µL of anti-HA bead slurry to a tube for each sample.

2 Wash the beads 3 times with KPBS on the magnetic rack.

3 Resuspend the beads in 50 µL KPBS.

Cell Lysis 10m

4 Aspirate the media from the cells.

5 Trypsinize the plate, quench with 10% FBS, and transfer to a 15 mL tube.

6 Spin 300 x g, 00:03:00 . 3m

7 Resuspend in 1 mL PBS and transfer to an Eppendorf tube.

8 Spin 300 x g, 4°C, 00:03:00 . 3m

9 Resuspend in 1 mL KPBS Buffer and transfer to a pre-cooled Douncer On ice .

10 Lyse with 30 strokes of the Douncer.

11 Transfer the sample to a new Eppendorf tube and clean the Douncer between each sample.

12 Spin 1000 x g, 4°C, 00:04:00 . 4m

13 Collect the post-nuclear supernatant.

14 Add 50 µL of the post-nuclear supernatant to 120 µL of RIPA Buffer.

15 Perform the BCA Gold assay on the RIPA Buffer-lysed post-nuclear supernatants.

16 Adjust each sample to 100 µg in 850 µL of KPBS.

17 Reserve the rest of the post-nuclear supernatant to run as an input fraction in downstream SDS-PAGE, using RIPA buffer to equalize the protein concentration between samples.

LysoIP 55m 30s

18 Add the 100 µg of sample to each tube of resuspended anti-HA beads.

19 Incubate the tube with rotation at 4 °C for 00:20:00 . 20m

20 Place the tube on the magnetic rack and remove the supernatant

21 Wash the beads three times with 1 mL KPBS, transferring the resuspended beads to a new tube on the first and third washes.

22 After the third wash, place the tube onto the magnetic rack and remove the supernatant.

23 Elute by adding 70 µL RIPA buffer to the tubes, gently vortexing to resuspend.

24 Place the tube on a at 4 °C for 00:30:00 . 30m

25 Briefly and softly (e.g. ~ 50 x g, 00:00:30) to remove any liquid that has collected in the cap. 30s

26 Use the magnetic rack to remove the supernatant from the beads, transferring to a new tube.

27 The eluate and input fractions can now be denatured by adding 1/3 of the fraction volume of 4X NuPAGE LDS Sample Buffer + 10% beta-mercaptoethanol and boiling at 95 °C for 00:05:00 . 5m

Protocol references

1. Abu-Remaileh M, Wyant GA, Kim C, Laqtom NN, Abbasi M, Chan SH, Freinkman E, Sabatini DM. Lysosomal metabolomics reveals V-ATPase- and mTOR-dependent regulation of amino acid efflux from lysosomes. Science. 2017 Nov 10;358(6364):807-813. doi: 10.1126/science.aan6298. Epub 2017 Oct 26. PMID: 29074583; PMCID: PMC5704967.