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Isolation of Lipid Droplets from Cultured Cortical Neurons

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We use this protocol and it's working

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Abstract

This protocol provides a robust method for purifying lipid droplets from cultured cortical neurons, suitable for lipidomic analysis of constituent lipids.

Guidelines

The protocol needs prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

Materials

- Cortical neurons: 8-10 × 15 cm dishes (~80% confluent) 14-21 DIV

KLH45 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**SML1998

- MEPS Buffer Components:

1. PIPES **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P6757
2. EGTA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E3889
3. Magnesium sulfate solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M3409
4. Potassium hydroxide ACS reagent, ≥85%, pellets **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**221473-500G

- cOmplete Protease Inhibitor Cocktail (Roche, 45-11697498001)
- Sucrose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S0389
- BODIPY™ 493/503 (4,4-Difluoro-1,3,5,7,8-Pentamethyl-4-Bora-3a,4a-Diaza-s-Indacene) **Thermo Fisher Catalog #**D3922
- CellMask™ Orange Plasma membrane Stain **Thermo Fisher Catalog #**C10045

Equipment:

- Nitrogen Cavitation Apparatus
- Centrifugation Equipment:

1. Table-top centrifuge (capacity 2 ml, up to 1800 g),
2. Ultracentrifuge with swinging-bucket rotor (SW41Ti, Beckman Coulter; up to 1,74,000 g)

- Needles and Syringes: 25 gauge needles with 2 ml syringes.

Neuronal Preparation

1 Isolate cortical neurons from P0-P1 pups and culture them in 15 cm dishes (8-10 plates).

2 Maintain the neurons in standard culture condition for 14-21 days to allow growth of axons and synapse formation.

3 Treat the neurons with [M] 5 micromolar (µM) KLH45 (dissolved in DMSO) for 24 hours prior to harvesting.

Harvesting and Lysis

4 Harvest neurons in 1.5 mL of [M] 0.9 Mass Percent MEPS buffer supplemented with 1X protease inhibitor cocktail.

MEPS buffer composition: PIPES: 35 mM (adjust pH to 7.2 with KOH), EGTA: 5 mM, $MgSO_4$: 5
mM, Sucrose: 0.9 M

5 Lyse the neurons using the nitrogen cavitation method or Isobiotec Cell Homogenizer (8-10 strokes with 16-micron clearance). Confirm complete lysis under an inverted white-field or phase-contrast microscope.

Post-Nuclear Supernatant (PNS) Preparation 10m

6 Centrifuge the lysate at 1800 x g, 4°C, 00:10:00 to remove unlysed somas, nuclei, and plasma membrane fractions. 10m

7 Collect the supernatant (called post-nuclear supernatant, PNS) into a fresh tube.

Gradient Setup and Ultracentrifugation 1h 30m

8 Pour the PNS over 2.5 M MEPS in a clear ultracentrifuge tube (SW41).

9 Overlay the bottom layer with 2 mL each of [M] 1.2 Mass Percent, [M] 0.5 Mass Percent, and [M] 0 Mass Percent MEPS to establish a step gradient.

10 Load the ultracentrifuge tube into the SW41Ti rotor and balance it against an identical tube.

11 Centrifuge the gradient at 174000 x g, 4°C, 01:30:00. 1h 30m

Lipid Droplet (LD) Collection

12 Carefully transfer the ultracentrifuge buckets to ice.

13 Collect the LDs floating on the top of gradient using a syringe fitted with appropriate needles (25 gauge) or a tube slicer.

Storage

14 Snap-freeze the collected LDs in liquid nitrogen for future use.

Validation of Purity

15 Perform western blotting to confirm the purity of the isolated LDs.

16 Confirm enrichment of LD by Perilipin-2 and contamination (if any) using organelle-specific markers for mitochondria, ER, Golgi, and endosomes.

Note

- Appropriate clearance/number of strokes with Isobiotec cell Homogenizer, or pressure during Nitrogen cavitation is critical for effective lysis while preserving organelle integrity.
- Extreme care is required during the gradient setup to prevent contamination of the LD layer.
- Ensure all ultracentrifuge tubes are properly balanced to avoid damage of rotor/centrifuge.
- Cell-Mask Plasma Membrane Stain can be used to confirm absence of unwanted membrane contamination in LDs samples.