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Clusterin Phospholipid Particles Flotation Assay Using an Optiprep Step Gradient

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We use this protocol and it's working

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Abstract

This protocol is based on Yeh FL et al. Neuron (2016) and details how to efficiently separate Clusterin phospholipid particles (Clusterin:DMPC) from free Clusterin together with binders by flotation using an Optiprep step density gradient.

Materials

Reagents

- PBS
- 2% Na-deoxycholate
- Trichloroacetic acid
- acetone
- OptiPrep Density Gradient Medium (Merck, D1556)
- 3.5 mL Thickwall Polycarbonate Tube (349622, Beckman Coulter)
- NuPAGE™ LDS Sample Buffer (4X) buffer (Thermo Fisher Scientific)

Equipments

SW55 Ti rotor (Beckman Coulter)
Bioruptor sonication bath (Diagenode)

OptiPrep™ Density Gradient Medium **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D1556)

3.5 mL, Open-Top Thickwall Polycarbonate Tube, 13 × 51mm - 25Pk **Beckman Coulter Catalog #**349622

Rhodanese Aggregation Assay

1 Perform the rhodanese aggregation reaction* in the presence of Clusterin or purified Clu:DMPC nanodiscs** at molar ratio D-Rhod:Clu 1:3.

Note

*See protocol 'Prevention of rhodanese aggregation assay'.
** See 'Formation and isolation of Clu-phospholipid particles'.
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2 Centrifuge the rhodanese aggregation reactions in the presence of Clusterin or Clu:DMPC at 20000 x g, 4°C, 00:15:00 to remove big aggregates.

15m

Note

NOTE: Here we use D-Rhodanese, but binding of other Clusterin substrates to Clusterin:DMPC
nanodiscs can be tested and isolated using this assay.

Optiprep Gradient

3 Mix the supernatant with OptiPrep Density Gradient Medium (Merck, D1556, 60% iodixanol) to a final concentration of 36% iodixanol in PBS and final volume of 1 mL in a 3.5 mL Thickwall Polycarbonate Tube (349622, Beckman Coulter).

4 Carefully place 1 mL of 24% iodixanol diluted in PBS on top of the 36% iodixanol layer.

5 Carefully place 1 mL of PBS as top layer.

6 Centrifuge the gradient at 54000 rpm, 4°C, 03:00:00 using a SW55 Ti rotor (Beckman Coulter).

3h

7 After centrifugation, carefully collect 6 fractions of 0.5 mL.

TCA Precipitation for SDS-PAGE Analysis

8 Dilute each fraction with 0.5 mL PBS.

9 Add 40 µL of 2% Na-deoxycholate and vortex.

10 Incubate On ice for 00:15:00. 15m

11 Add 100 µL of 100% Trichloroacetic acid and vortex.

12 Incubate On ice for 01:00:00. 1h

13 Centrifuge the samples at 20000 x g, 4°C, 00:30:00. 30m

14 Add 500 µL of ice-cold acetone.

15 Vortex or sonicate in a Bioruptor sonication bath (Diagenode) (2 cycles of 00:00:30 on – 00:00:30 off) for faster resuspension.

16 Centrifuge at 200000 x g, 4°C, 00:10:00. 10m

17 Air dry the pellets.

18 Add 30 µL [M] 300 millimolar (mM) Tris pH 8.8.

19 Incubate On ice for 00:05:00 . 5m

20 Add 30 µL NuPAGE™ LDS Sample Buffer (4X) buffer (Thermo Fisher Scientific) containing [M] 100 millimolar (mM) DTT and boil the samples for 00:10:00 . 10m

21 Separate the proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblotting.

Note

NOTE: Isolated Clusterin phospholipid particles bound to a substrate can be also used for further experiments like cellular uptake, see Yeh FL *et al. Neuron* (2016).

Protocol references

Yeh FL, Wang Y, Tom I, Gonzalez LC, Sheng M. TREM2 Binds to Apolipoproteins, Including APOE and CLU/APOJ, and Thereby Facilitates Uptake of Amyloid-Beta by Microglia. Neuron. 2016 Jul 20;91(2):328-40. doi: 10.1016/j.neuron.2016.06.015. PMID: 27477018.