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Protocol status: Working
We use this protocol and it's working

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Formation and isolation of Clu phospholipid particles

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ABSTRACT

This protocol details how to efficiently make *in vitro* and isolate Clu-phospholipid particles using purified Clusterin from HEK293E cells (dx.doi.org/10.17504/protocols.io.bvvkn64w) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC).

ATTACHMENTS

976-2533.docx

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MATERIALS

NativePAGE 3%–12% Bis-Tris SDS gel **Thermo Fisher Scientific Catalog #BN1001BOX**

NativePAGE™ Sample Buffer (4X) **Thermo Fisher Catalog #BN2003**

NativePAGE™ Running Buffer (20X) **Thermo Fisher Catalog #BN2001**

InstantBlue® Coomassie Protein Stain (ISB1L) (ab119211) **Abcam Catalog #119211**

Equipment

Glass vial

NAME

Waters Corporation

BRAND

186000272C

SKU

https://www.waters.com/nextgen/in/en/shop/vials-containers--collection-plates/186000272c-lcgc-certified-clear-glass-12-x-32-mm-screw-neck-vial-with-cap-a.html

LINK

Formation of Clu-phospholipid particles

17h 30m

1 Prepare 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) stock solution: 5-25 mg/ml DMPC in 3:1 Chloroform:Methanol and store it at -80 °C.

2 Transfer the corresponding amount of DMPC solution to a glass vial and remove the solvent by evaporation through a constant stream of nitrogen gas until it dries out and becomes waxy.

Note

Avoid overdrying the lipids (extensive white film). If it happens, the lipids can be dissolved in 3:1 Chloroform:Methanol and dried again.

3 Resuspend the lipids with 1x PBS pH 7.2 to obtain the desired concentration, vortex and sonicate in a Bioruptor sonication bath (Diagenode) (25 cycles of 5 seconds on – 5 seconds off), or similar. The resulting mixture is turbid and white.

4 Mix [M] 10 micromolar (µM) Clusterin with [M] 10 millimolar (mM) DMPC in a PCR tube for a Clusterin:DMPC ratio 1:1000. For example, 10 µL Clusterin [M] 20 micromolar (µM) + 10 µL DMPC [M] 20 millimolar (mM) in PCR tubes.

Note

1:1000 Clusterin:DMPC ratio results in extensive Clusterin lipidation. The ratio can be increased and the reaction can be scaled up to obtain high amounts of Clu-phospholipid particles, e.g. for further isolation by size exclusion chromatography.

5 Incubate the sample through 3 cycles of 18 °C for 15 minutes – 30 °C for 15 minutes using a thermocycler. 1h 30m

5.1 Incubate the sample at 18 °C for 00:15:00 – 30 °C for 00:15:00 using a P thermocycler (1/3). 30m

5.2 Incubate the sample at 18 °C for 00:15:00 – 30 °C for 00:15:00 using a P thermocycler (2/3). 30m

5.3 Incubate the sample at 18 °C for 00:15:00 – 30 °C for 00:15:00 using a P 30m thermocycler (3/3).

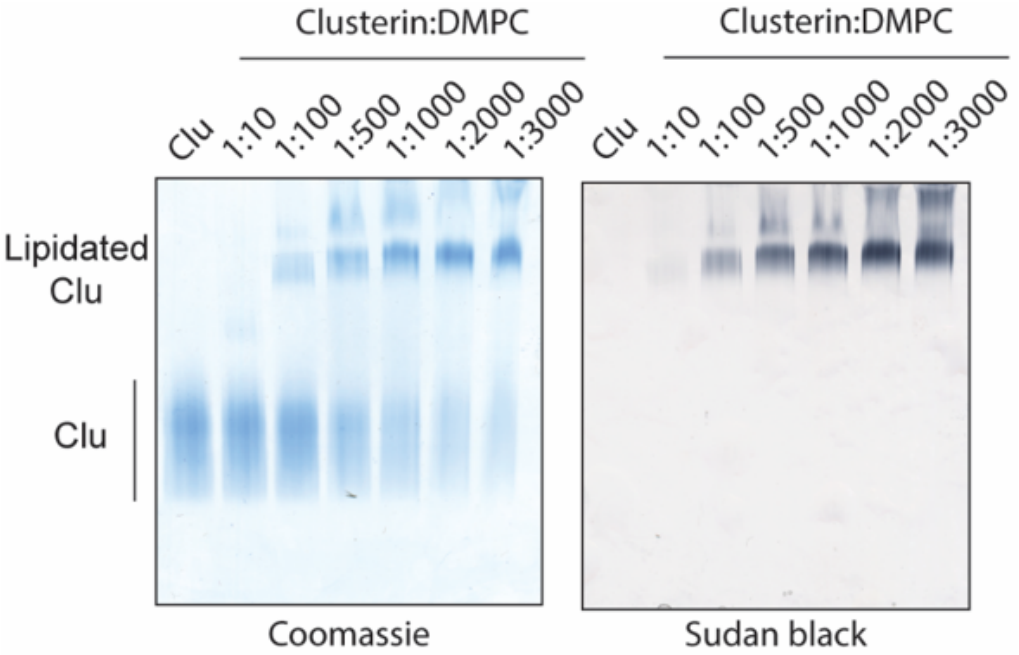
6 Analyze Clusterin lipidation by Native polyacrylamide gel electrophoresis (Native-PAGE). Mix the samples with NativePAGE Sample Buffer (4X) (refer materials section), load them on a NativePAGE 3%–12% Bis-Tris SDS gel and run the gel in NativePAGE running buffer (refer materials section) at 140 V.

Note

Analysis of protein staining (Coomassie) and lipid staining (Sudan black B) should be done in independent gels.

7 For protein staining, incubate the gel Overnight with InstantBlue and de-stain next day with water. 8h

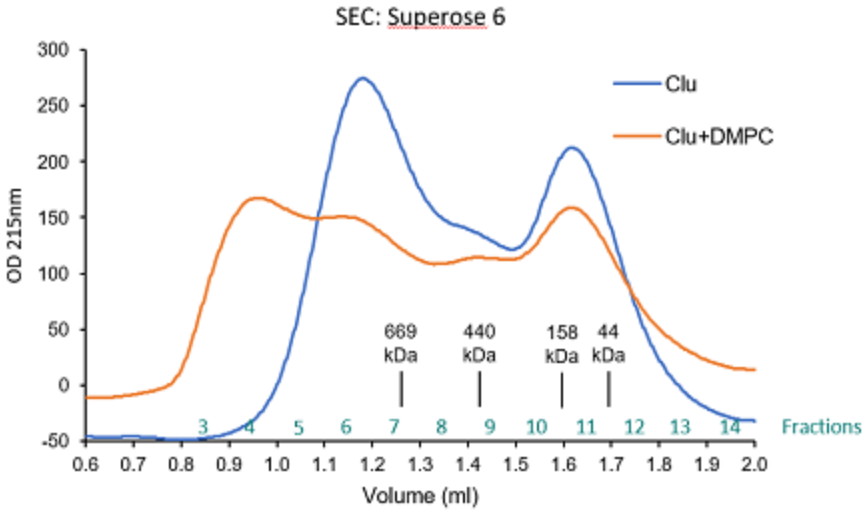
8 For lipid staining, incubate the gel Overnight with 0.4% Sudan black B (MERCK, S0395) in 16.7% acetone, 12.5% acetic acid solution (previously centrifuged to remove precipitates) and de-stain the next day with 20% acetone, 15% acetic acid. 8h

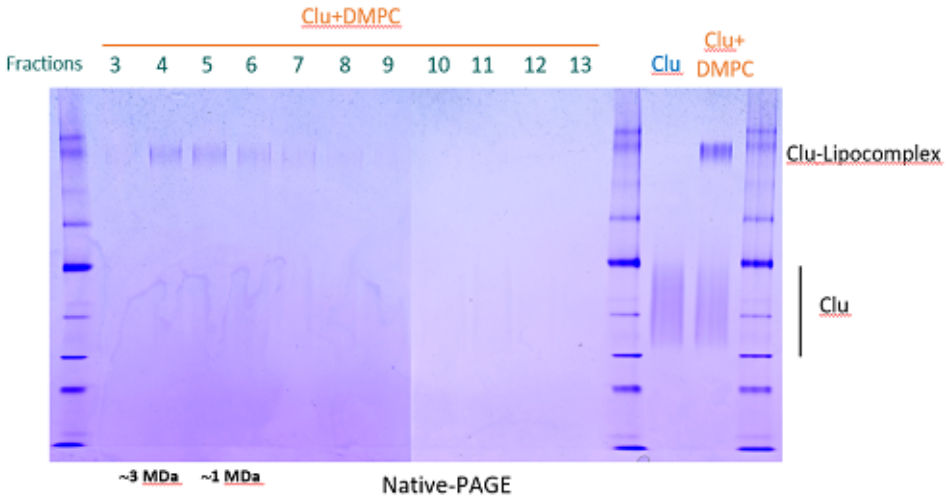


Isolation of Clu-phospholipid nanodisc complexes

9 Centrifuge lipidated Clusterin using a table top centrifuge for 30 seconds to pellet big multi-lamellar lipid vesicles (white pellet).

10 Load the supernatant into a Superose 6 previously equilibrated with 1x PBS. Clu-phospholipid nanodisc complexes elute in the first fractions after void volume.





11 Collect and concentrate the Clu-phospholipid complex containing fractions by ultrafiltration using Vivaspin MWCO 10.000 (GE Healthcare).

Note

Even after isolation of Clu-phospholipid particles, some free Clusterin impurity is observed likely due to a dynamic exchange between free and lipidated Clusterin.