**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).

**Keywords:** Clusterin, apolipoprotein J, DMPC, lipidation.

Formation of Clu-phospholipid particles

1.- Prepare1,2-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 (Waters, 186000272C) 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 10 µM Clusterin with 10 mM DMPC in a PCR tube for a Clusterin:DMPC ratio 1:1000. For example, 10 µL Clusterin 20 µM + 10 µL DMPC 20 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 PCR thermocycler.

6.- Analyze Clusterin lipidation by Native polyacrylamide gel electrophoresis (Native-PAGE). Mix the samples with NativePAGE Sample Buffer (4X) (Thermo Fisher Scientific, BN2003), load them on a NativePAGE 3%–12% Bis-Tris SDS gel (Thermo Fisher Scientific, BN1001BOX) and run the gel in NativePAGE running buffer (Thermo Fisher Scientific, BN2001) 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 (Abcam, ab119211) and de-stain next day with water.

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.

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.

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10.- 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.

**Relevant references**

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