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Methanol-Chloroform-Water Precipitation

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1 Works for me dx.doi.org/10.17504/protocols.io.biknkcve

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ABSTRACT

Methanol-chloroform-water (MCW) precipitation is a rapid method for removing interferring species from protein samples prior to LC-MS analysis. MCW precipitation can effectively remove both ionic and non-ionic detergents and salts. Precipitating less than 1 µg protein is challenging yet possible with careful pipetting.

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MATERIALS TEXT

MATERIALS

Protein LoBind 1.5mL microcentrifuge tubes **Eppendorf Catalog #0030108116**

Water Optima™ LC/MS Grade Fisher Scientific **Contributed by users Catalog #10728098**

Pierce™ Formic Acid **Thermo Fisher Catalog #TS-28905**

Methanol Optima™ LC/MS Grade Fisher Chemical **Fisher Scientific Catalog #A456-4**

Acetonitrile Optima™ LC/MS Grade Fisher Chemical **Fisher Scientific Catalog #A955-500**

Chloroform (HPLC grade) **Sigma Aldrich**

Resuspension buffer: 94.9% water, 4.9% acetonitrile, 0.2% formic acid

SAFETY WARNINGS

Halogenated solvent waste should be disposed properly as it poses environmental hazards.

1 Dilute protein sample to 150 µL with Optima-grade water.

2 Precipitate protein at interface

2.1 Add 600 µL Optima-grade methanol and mix well.

2.2 Add 150 µL HPLC-grade chloroform and mix well.

2.3 Centrifuge **20000 x g, 4°C, 00:10:00** and discard top layer while being careful to not disturb interface between layers. Use a gel-loading tip to remove last bit.

3 Pellet protein at interface

3.1 Add 600 µL and homogenize the solvent, being careful not to break up the precipitated protein.

3.2 Centrifuge **20000 x g, 4°C** and discard supernatant, being careful not to disturb pellet.

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4 Wash pellet with methanol. (This step can be repeated if a high level of contaminating species is anticipated)

4.1 Add 600 µL methanol and mix by inverting tube.

4.2 Centrifuge **20000 x g, 4°C** and discard supernatant, being careful not to disturb pellet.

5 Redissolve protein in LC buffer

5.1 Evaporate excess methanol by laying tube on its side for 5 min at room temperature.

5.2 Redissolve precipitated protein in 30 µL Buffer A. Incubate 10 min on ice. If protein is not completely dissolved, sample can be briefly sonicated with a bath sonicator.

5.3 Centrifuge **20000 x g, 4°C** and transfer supernatant to LC sample vial.

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