MAR 30, 2021

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.biknkcve

Protocol Citation: Bryon Drown, Kelleher KRG KRG Research Group 2021. Methanol-Chloroform-Water Precipitation. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.biknkcve>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Jul 15, 2020

Last Modified: Mar 30, 2021

PROTOCOL integer ID:
 39278

Keywords: protein, mass spectrometry, top-down proteomics

Methanol-Chloroform-Water Precipitation

Bryon Drown¹, Kelleher KRG KRG Research Group²

¹Purdue University; ²Northwestern University

Kelleher Research Group

Tech. support email: kelleher-ofc@northwestern.edu

Kelleher KRG KRG Research Group

Northwestern University, National Resource for Translational...

ABSTRACT

Methanol-chloroform-water (MCW) precipitation is a rapid method for removing interfering 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.

MATERIALS

MATERIALS

⊗ Protein LoBind 1.5mL microcentrifuge tubes **Eppendorf Catalog #003010811**

⊗ 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.

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

1

2 Precipitate protein at interface

2.1 Add 600 μ L Optima-grade methanol and mix well.

2.2 Add 150 μ L 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 μ L 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.

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.