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

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BAF_Protocol_007a Chloroform/Methanol Precipitation

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ABSTRACT

This protocol extends BAF_Protocol_007 as an alternative to acetone precipitation (normal method). The chloroform/methanol precipitation is a better method if the sample is high in lipid or other hydrophobic small molecules. This method also produces a protein pellet and not the normal liquid interface and as such is easier.

GUIDELINES

This precipitation would be substituted in for the acetone precipitation normally used in BAF_Protocol_007.

MATERIALS

Pre-cleaned microtubes 1.5 mL - SEAL-RITE‰ 1.5 ML MICROCENTRIFUGE TUBES color: natural, USA Scientific
Pipette tips - Fisher Brand, yellow, part number: 02-681-151
ABC - Fluka analytical Ammonium Bicarbonate, Sigma Aldrich, 09830-500G
VWR Analog Vortex mixer - CAT No: 58816-121
Centrifuge 5427 R, Eppendorf
Water - Fisher Chemical, W6-4, Optima LC/MS
Methanol - A456-212, Methanol, optima LC/MS
Chloroform - AC423550010, ACS Grade 99.8+%

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Keywords: protein precipitation, mass spectrometry, sample cleanup, lipds

Chloroform/Methanol PPT

28m

1 100 uL of protein extract of sample added to Eppendorf tube. 1m

2 400 uL of Methanol was added, vortexed well. 2m

3 200 uL of Chloroform was added, vortexed well. 2m

4 300 uL of Water was added, vortexed well. 2m

5 Sample was centrifuged at 13,000 rpm for 2 minutes 4m

6 The upper layer was carefully removed (not disturbing the protein interface). 1m

7 300 uL of Methanol was added, vortexed well. 2m

8 Samples were centrifuged at 13,000 rpm, for 2 minutes. 4m

9 Supernatants were discarded and the protein pellet was air dried. Resuspend in 100 mM Ammonium Bicarbonate in volume based on protein amount. 10m