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BAF_Protocol_007a Chloroform/Methanol Precipitation

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Protocol status: Working

We use this protocol and it's working

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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+%



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