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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+%



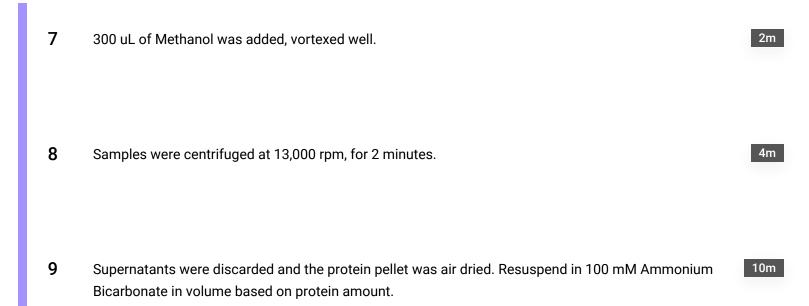
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Keywords: protein precipitation, mass spectrometry, sample

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



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