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Proteomics Sample Preparation for Affinity-Purification Mass Spectrometry

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We use this protocol and it's working

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Abstract

Protocol for the preparation of immunoprecipitated protein samples captured via magnetic bead technologies, for affinity-purification mass spectrometry analysis.

Materials

Acetonitrile ≥99.9% VWR Catalog#1.00030.2500
Formic Acid, 99.0+%, Optima™ LC/MS Grade ThermoFisherScientific Catalog#A117-50
Water, for HPLC-MS FisherScientific Catalog#10777404
EPA Screw Vial Assembled Kit, 20mL amber glass EPA Thermo Scientific Catalog#11543750
Sodium Dodecyl Sulfate (SDS), Micropellets ThermoFisherScientific Catalog#15450685
Hepes 99.6%, Formedium Catalog#HEPES10
Bond-Breaker™ TCEP Solution, Neutral pH ThermoScientific Catalog#77720
Iodoacetamide, BioUltra SigmaAldrich Catalog#I1149-25G
Trifluoroacetic acid (TFA) SigmaAldrich Catalog#302031-100ML-M
Methanol ThermoFisherScientific Catalog#10653963
Triethylammonium bicarbonate buffer (TEABC) SigmaAldrich Catalog#T7408-500ML
Trypsin/Lys-C Mix ThermoFisherScientific Catalog#15956915
n-Dodecyl-beta-Maltoside Detergent (DDM) ThermoScientific Catalog#89902
S-Trap micro columns Protifi Catalog#C02-micro-80
Complete EDTA-free protease inhibitor cocktail Roche Catalog#11873580001
PhosSTOP phosphatase inhibitor cocktail tablets Roche Catalog#4906837001
Micro Tube 2ml Low Binding Sarstedt Catalog#72.695.600
Eppendorf twin.tec PCR Plates LoBind (96-well) ThermoFisherScientific Catalog#15280735.

Note

All solvents and buffers were prepared in or aliquoted from glass amber vials, using sterile (not autoclaved) pipette tips or glass cylinders.

Equipment:

ThermoMixer C (Eppendorf)
Savant SpeedVac SPD140DDA Vacuum Concentrator
(ThermoScientific)
Micro Star 17 microcentrifuge (VWR)
Invitrogen DynaMag-2 Magnet (rack)

Protein Elution and Preparation

1 Allow samples to thaw at room temperature for 15 minutes.

2 Add 40µl SDS lysis buffer: 2% SDS (w/v), 20mM HEPES pH 8, with complete EDTA-free protease inhibitor cocktail (Roche) and PhosSTOP phosphatase inhibitor cocktail tablets (Roche), to bead slurry and mix by vortex for 15 seconds.

3 Place sample tubes into a magnetic rack and leave for 2-3 minutes to allow for separation of magnetic beads from supernatant.

4 Carefully remove the supernatant containing eluted protein, taking care not to disturb magnetic beads, and pipette into a fresh 2ml microtube.

5 Reduce samples with 10mM TCEP (final concentration, 100mM TCEP stock in 300mM TEABC) for 30 minutes, 60°C at 1100rpm (ThermoMixer C, Eppendorf).

6 Alkylate samples in the dark with 40mM iodoacetamide (final concentration, 400mM stock) for 30 minutes at 25°C at 1100rpm (ThermoMixer C, Eppendorf).

7 Add 20% SDS to achieve a final concentration of 5% SDS, then acidify samples by addition of Trifluoracetic acid to a final concentration of 1%.

Micro S-Trap On-Column Digest

8 Add 6x sample volume of wash buffer (90% methanol, 10% 1M TEABC).

9 Mix sample by pipetting up and down, then load 150µl of sample onto micro-columns inside fresh 2ml Protein LoBind Eppendorf tubes (Sarstedt) within a centrifuge.

10 Centrifuge at 1000g for 1 minute and discard flow-through.

11 Repeat steps 14 and 15 until all sample has been loaded through micro-column.

12 Wash S-Trap columns (with bound protein) by adding 150µl wash buffer followed by centrifugation at 1000g, 1 minute. Discard flow-through. Repeat a further 3x.

13 Digest protein by addition of 40µl (1µg) of Trypsin/Lys-C mix (MS grade, Promega, UK) in 50mM TEABC solution (pH 8) at 47°C for 1 hour and 20 minutes, followed by incubation at 22°C overnight (approximately 16 hours).

Peptide Elution

14 Elute samples by addition of 40µl 50mM TEABC (pH 8) and centrifuge at 1000g for 1 minute.

Note

Do NOT discard flow-through containing peptides at any step of elution.

15 Add 40µl 0.15% (v/v) formic acid (FA) and centrifuge at 1000g for 1 minute.

16 Add 40µl 80% acetonitrile (ACN), 0.15% FA and centrifuge at 1000g for 1 minute. Repeat twice (3x total).

17 Dry peptides at room temperature using a vacuum centrifuge and store dried peptides at -20°C until mass spectrometry analysis.

Peptide Resuspension

18 Thaw peptide samples at room temperature for 15 minutes, followed by centrifugation at 17,000g for 15 seconds.

19 Resuspend samples by addition of 50µl 0.1% formic acid supplemented with 0.015% N-Dodecyl-B-D-Maltoside (DDM) and mix at 1800rpm for 30 minutes at room temperature (ThermoMixer C, Eppendorf).

20 If analysing immediately, transfer 20µl of each sample to a 96-well plate or LC-MS vials and inject ~200ng peptides per sample for MS analysis.