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BAF_Protocol_002 On-Bead Digestion (Magnetic Beads) of Proteins in Solution or Provided Already on Beads

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

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Abstract

This protocol is used to more easily digest a tissue or cell protein lysate (after precipitation and reconstitution) on a magnetic bead for easier manipulation and washing. The protocol from step 13 on would be used for proteins already captured on a magnetic beads such as antibody (IP) or avidin coated - usually as an enrichment step. The proteins are easily washed to remove contaminants such as detergents or salts. The resulting peptide mixture can be further purified using a C18 tip such as our Protocol 003.

Guidelines

If using other types of magnetic beads for capture (such as antibody for IP or Avidin to pull out biotin tagged proteins), make sure that the amount of beads is titrated to the amount of starting proteins to ensure the beads are fully coated. Otherwise, the digestion will release the capture protein (antibody or avidin).

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.
Micropipettes
ABC - Fluka analytical Ammonium Bicarbonate, Sigma Aldrich, 09830-500G
DTT - Fisher Bioreagents Dithiothreitol, C4h10o2s2 F.W. 154.24
Iodoacetamide - Sigma Aldrich 1149-5G PCode:1002138224
Promega Trypsin: Sequencing grade modified, frozen, V511C, Promega
FA - Fisher chemical A117-50, Formic Acid, optima LC/MS
ACN - Fisher chemical A955-4, Acetonitrile, optima LC/MS
Water - Fisher Chemical, W6-4, Optima LC/MS
DMSO - Sigma D8418
Ethanol - Sigma E7023
Syringe - Unimetrics PKS250, 250µL Peek Laboratory Syringe
Magnetic beads A- GE Healthcare Sera-Mag™ Speed bead magnetic carboxylate modified particle 15mL azide 0.05%, part number: 45152105050250
Magnetic beads B- GE Healthcare Sera-Mag™ Speed bead magnetic carboxylate modified particle 15mL azide 0.05%, part number 65152105050250
Magnetic rack - MR02
2 to 20 µL Micropipette - Gilson™ F144056MT
10 to 100 µL Micropipette - Gilson™ F144057MT
20 to 200 µL Micropipette - Gilson™ F144058MT
100 to 1000 µL Micropipette - Gilson™ F144059MT
VWR Analog Vortex mixer - CAT No: 58816-121

Before start

REAGENTS: (All reagents to be prepared fresh for each digestion)
1. 100 mM ammonium bicarbonate (ABC): 0.158 g in 20 mL distilled water
2. 50 mM ABC: 0.079g in 20 mL distilled water
3. Acetonitrile (ACN)
4. Ethanol
5. 100 mM DTT: 0.0015g in 100 uLof 100mM ABC (DO NOT mix until directly before you are ready to use)
6. 500 mM Iodoacetamide: 0.01g in 100 uL of 100 mM ABC (DO NOT mix until directly before you are ready to use)
7. Trypsin solution: Keep on ice. Promega (cat. # V5113) is already diluted in 50 mM acetic acid.
8. DMSO 2% in water.

Prepare Stock Solution of Beads (for Tissue or Cell Lysates) 23m

1 Remove both beads (A and B) from the fridge and keep them at room temperature for 10 minutes. Combine 20 uL of magnetic beads A and 20 uL of magnetic beads B.- 11m

2 Add 160 uL of water (LC-MS/MS grade). 30s

3 Place tubes with beads on a magnetic rack and let beads settle for 2 minutes. Remove and discard supernatant. 2m 30s

4 Rinse beads with 200 uL of water by pipette mixing (off the magnetic rack). Place tubes with beads on a magnetic rack and let beads settle for 2 minutes. Remove and discard supernatant. 3m

5 Repeat step 4 two times. 6m

6 Add 100 uL of water to beads, 10ug/uL stock bead mix, and store in the fridge. Prepared beads can be stored at 4ºC indefinitely. Never freeze the beads. 1m

On-bead Digestion (Start Step #13 if Beads were Affinity Capture) 17h 45m

7 To 10 ug of total protein add 2 uL of bead mix (20 ug) from step 6 in the total volume of 200 uL FA 0.1% (aim for a pH ~2). 2m

8 Add 200 uL of ACN (100% stock) to reach a final concentration of 50% (v/v). Incubate for 8 minutes at room temperature. 9m

9 Place the tubes on a magnetic rack for 2 minutes. Remove and discard supernatant. 3m

10 While on the magnet rack, rinse beads with 200 µl of 70% absolute ethanol, incubate for 30 s, and discard supernatant. 2m

11 Repeat step 10 once. 2m

12 Rinse beads with 180 µl of 100% ACN, incubate for 30 s, and discard supernatant. 1m

13 Add 20 uL of 100 mM ABC buffer. If using beads that already have proteins captured such as IP (antibody), Biotin, etc, wash the beads three times with 200 ul ABC buffer to remove previous buffer and then add 20 uL ABC. 1m

14 Reduce the proteins by adding 2 µL of 100 mM DTT to the beads, incubate for 30min at room temperature. 30m

15 Alkylate the proteins by adding 2 µL 500 mM iodoacetamide at room temperature for 30 min in dark. 30m

16 Dilute the sample with 20 µL of water (final ABC concentration to 50 mM). Check the sample pH it should be around 8-7. 5m

17 Add 2.5-5 µL of Promega trypsin on ice to each sample for about 1 µg of trypsin. 1m

18 Incubate overnight at 37ºC, 700 rpm. 16h

19 Add 1000 uL of ACN 100% to final concentration > 95%. Incubate for 8 minutes at room temperature. 9m

20 Place the tubes on a magnetic rack for 2 minutes. Remove and discard supernatant. 3m

21 Rinse beads with 180 µl of 100% ACN, incubate for 30 s, and discard supernatant. 2m

22 Add 10 uL of 2% DMSO (in water, not acidic). Give a quick spin (~2 seconds) in a bench-top centrifuge to aid the liquid removal from the tube walls. 1m

23 Place tubes on a magnetic rack for 2 minutes and recover supernatant making sure to not recover any beads. 3m

24 Dilute the samples with 0.1% FA to <1% DMSO and go to BAF_Protocol_003 - Dessalting with C-18 tips. 1m

Protocol references

Natalia Gabrielli, Christoniki Maga-Nteve, Eleni Kafkia, Mandy Rettel, Jakob Loeffler, Stephan Kamrad, Athanasios Typas, Kiran Raosaheb Patil, Unravelling metabolic cross-feeding in a yeast–bacteria community using C-based proteomics, Molecular Systems Biology, 10.15252/msb.202211501, **19**, 4, (2023).