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BAF_Protocol_006 On-Bead Peptide Cleanup (Digested by Other Method)

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Protocol status: Working

We use this protocol and it's working

Created: February 22, 2024



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Protocol Integer ID: 95559

Keywords: digestion, sample cleanup, beads, mass spectrometry, bead peptide cleanup, using bead, peptide, example solution digest, bead, mag bead, downstream mass spectrometry, mass spectrometry, cleanup step, protein, removal of salt, protocol

Abstract

This protocol is for using beads as a cleanup step after protein is digested using a different method (not directly on Sera-Mag beads), for example solution digest. The peptides are precipitated onto the beads to allow for removal of salts and detergents that will interfere with downstream mass spectrometry.

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.

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

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



On-Bead Clean-up

20m

- 1 Stock Solution of magnetic Sera-Mag beads is made in section 1 of BAF_Protocol_004 and can be stored at 4C indefinitely and used as needed (never freeze).
- 2 Peptide digest (for example from a solution or in-gel digest) should be reduced in volume so that a 95% ACN (acetonitrile) final concentration can be made within the Eppendorf tube volume. This will precipitate the peptides onto the beads for clean-up. See next steps.
- 3 To the peptide digest that has been reduced in volume, add 2uL of bead stock mix (20ug). 1m
- 4 Add 100% ACN to a final concentration of 95% ACN. Incubate for 8 minutes at room temperature. 9m
- 5 Placed tubes on a magnetic rack for 2 minutes. Remove and discard supernatant. 3m
- 6 Rinse beads with 180uL of 100% ACN, incubate for 30s then remove and discard supernatant. 2m
- 7 Add 10uL of 2% DMSO in water. Give quick spin (2-3s) in bench-top centrifuge to aid liquid removal from tube walls to bottom of tube. 1m
- 8 Place tubes in magnetic rack for 2 minutes and recover supernatant making sure not to recover any beads. 3m
- 9 Dilute samples with 0.1% FA (formic acid in water) to <1% DMSO. Further clean-up using C18 tips may be done using BAF_Protocol_003. 1m