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

nesf[1]

[1]UVA Biomolecular Analysis Facility Core

Biomolecular Analysis Facility

nesf

FEB 23, 2024

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.n92ldmkknl5b/v1

Protocol Citation: nesf 2024. BAF_Protocol_006 On-Bead Peptide Cleanup (Digested by Other Method) . **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldmkknl5b/v1

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

Created: Feb 21, 2024

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

Last Modified: Feb 23, 2024

PROTOCOL integer ID: 95559

Keywords: digestion, sample cleanup, beads, mass spectrometry

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