Feb 01, 2024 Version 1

BAF_Protocol_003 Desalting V.1

DOI
dx.doi.org/10.17504/protocols.io.36wgqjzmyvk5/v1

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Protocol Citation: Nicholas Sherman 2024. BAF_Protocol_003 Desalting. **protocols.io https://dx.doi.org/10.17504/protocols.io.36wgqjzmyvk5/v1**

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

Created: April 11, 2023

Last Modified: May 05, 2025

Protocol Integer ID: 80342

Keywords: mass spectrometry, digestion, sample cleanup, C18, baf-protocol-003, procedure salt, end of the procedure salt, peptides from solution, gel digestion, analytical column life on the mass spectrometer, mass spectrometer, peptide, procedure

Abstract

The procedure is used to desalt and purify peptides from solution or in-gel digestion. This step results in minimal losses but increases analytical column life on the mass spectrometer. At the end of the procedure salts and small molecules should be washed away from peptides.

Guidelines

Note 1: For optimal flow and peptide recovery, do not introduce air through the tip at any time during the procedure.

Note 2: This protocol is for C18 tips from Thermo Scientific. Pierce™ C18 Spin Tips & Columns part number for 10 µL tips 87782 and part number for 100 µL tips 87782.

Materials

ACN - Fisher chemical A955-4, Acetonitrile, optima LC/MS
FA - Fisher chemical A117-50, Formic Acid, optima LC/MS
Screw cap tubes - Fisher brand 02-681-339
0.5 mL tubes - SEAL-RITE® 0.5 ML MICROCENTRIFUGE TUBES color: natural, USA Scientific.
C18 tips 10 µL - Pierce™ C18 Spin Tips & Columns, part number 87782. Thermo Scientific.
C18 tips 100 µL - Pierce™ C18 Spin Tips & Columns, part number: 87784. Thermo Scientific.
10 µL tips - Finn Tip, part number: 9400300, Thermo Fisher.
Syringe - Unimetrics PKS250, 250µL Peek Laboratory Syringe
200 µL tips - Fisher Brand, yellow, part number: 02-681-151.
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

Troubleshooting

Before start

Amount of total peptide to choose between C18 10 µL or 100 10 µL tips:

10 µL tip can bind up to 8 µg of total peptide.

100 µL tip can bind up to 80 µg of total peptide.

Material Preparation

- Acidic solution: 2.5% FA
- Wetting solution: 70:30 ACN:water; 20 µL or 200 µL per sample
- Equilibration solution: 0.1% FA in water; 20 µL or 200 µL per sample
- Rinse solution: 0.1% FA in 5% ACN:water; 20 µL or 200 µL per sample
- Elution solution: 0.1% FA in 70% ACN:water for ESI-MS, up to 100 µL per sample

Procedure for the 10 µL C18 Tips

2h 11m

1 Set the micropipette to 10 µL and secure the pipette tip tightly to the end for optimum tip-to-micropipette seal and sample aspiration. 1m

2 Resuspend the sample with 0.1% FA (formic acid) or adjust sample to 0.1-1.0% FA using 2.5% FA to 12 µL total volume. Check that pH ~3.0. Needs to be pH 3 or slightly lower for efficient binding. 1m

3 Wet tip by aspirating 10 µL of 70% ACN (acetonitrile) in water and then discarding solvent. Repeat 5 times. 1m

4 Equilibrate tip by aspirating 10 µL of 0.1% FA and discarding solvent. Repeat 5 times. 1m

5 Aspirate up to 10 µL of sample (prepared in Step 2) onto the C18 tip. For the maximum efficiency, dispense and aspirate the sample for 30 cycles (in the sample tube - slowly). 2m

6 Rinse the tip by aspirating 10 µL of 0.1% FA with 5% ACN and discarding solvent. Washing out salts and other small molecules that do not stick to C18. Repeat 5 times. 1m

7 Elute the sample: Slowly aspirate 5-10 µL of 0.1% formic acid in 70% ACN and dispense into a pre-cleaned 0.6 mL tube. Dispense and aspirate in the tube 30 cycles. 1m

8 Rinse the tip by aspirating the 10 µL of 0.1% FA with 5% ACN and discarding solvent. Repeat 5 times. Same as step 4. 1m

9 Repeat steps 5-7. Second elution will be in same tube as first elution. 2m

10 Dry eluted sample in a speedvac and store at -80C until MS analysis. If going directly to MS analysis, then add 20-40uL 0.1% FA to tube, vortex, microfuge for 15 minutes at max speed, and take off 10uL into autosampler vial (do not touch bottom of tube). 2h

Procedure for the 100 µL C18 Tips

11 For larger tip (higher capacity) change volumes to 100uL with elution using 50-100uL. Final reconstitution volume will also be adjusted.