

KMS desalting protocol using Pierce Peptide Desalting Spin Columns (Thermo, 89851 and 89852)

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Pierce Peptide Desalting Spin Columns Protocol for LC-MS/MS

Add 100% Formic acid (FA) to sample to final concentration 2% v/v before proceeding with the protocol.

Solution Preparation

- Wetting solution: 100% ACN
- Washing solution: 0.5% FA
- Elution solution: 50% ACN, 0.5% FA

Use MQ Water to prepare solutions! Use clean glass bottles to store solutions (do not use plastic). Bottles should be brand new, washed only with MQ water and NEVER before exposed to detergents, or any other chemicals!!!!

Wet the Spin Columns

1. Remove the white tip from the bottom of the column and discard. Place the column into a **2 mL** microcentrifuge tube. Centrifuge at 5000 x g for 1 minute to remove the solution and pack the resin material. Discard the liquid.
2. Remove the top screw cap and load 300 μ L of Wetting solution into the column. Replace the cap, place the spin column back into a **2 mL** microcentrifuge tube and centrifuge at 5000 x g for 1 minute. Discard the liquid and repeat once.
3. Wash the spin column with Washing solution as described in step 2. Repeat 3 times.

Sample Binding

4. Place the column into a **2 mL** microcentrifuge tube. Load 300 μ L of the sample onto the column, replace the top cap and centrifuge at **3000 x g** for 1 minute. Discard the flowthrough. Repeat this step until all the sample is loaded on the column.

Washing

5. Place the column into a **2 mL** microcentrifuge tube. Load 300 μ L of Washing solution onto the column, replace the top cap and centrifuge at **3000 x g** for 1 minute. Discard the flowthrough. Repeat 3 times.

Eluting

6. Place the column into a **new 2 mL** microcentrifuge tube. Load 300 μ L of Elution solution onto the column, replace the top cap and centrifuge at **3000 x g** for 1 minute to collect the elute.
7. Place the column into **another new 2 mL** microcentrifuge tube. Apply another 300 μ L of Elution solution and centrifuge at **3000 x g** for 1 minute.
8. Combine the elute from step 7 and step 8 into a same **2 mL** microcentrifuge tube. Dry sample in SpeedVac and store dried peptides at -20C.

This step will be done by KMS:

Before LC-MS re-suspend sample in 10-20uL 0.1% Formic acid (pipette and vortex), after this remove insoluble particulates with 15min/15000RPM tabletop centrifuge spin, transfer supernatant into clean HPLC vial.