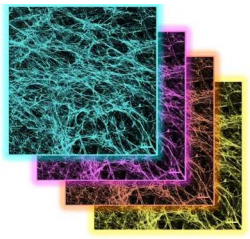
May 23, 2023 Version 2

Desalting of Peptides to Prepare for Mass Spectrometry Analysis V.2



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James Considine[1], Ikram Isa[1], Alexandra Naba[1]
[1]University of Illinois Chicago

Human BioMolecular Atl... The Matrisome Project

Alexandra Naba
University of Illinois Chicago

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We use this protocol and it's working!

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Abstract

Prior to proteomic analysis, peptide samples are desalted and eluted with freshly prepared 50% acetonitrile, 0.1% trifluoroacetic acid, followed by concentration in a vacuum concentrator. Peptides are then resuspended in freshly prepared 5% acetonitrile, 0.1% formic acid.

Note

- The last step can be conducted at a mass spectrometry facility according to their own preferred methods.
- After desalting, the concentration of the peptide solution can be measured by spectrophotometry.

Materials

Materials:

1. HPLC-grade water **Thermo Fisher Scientific Catalog #**51140
2. Trifluoro-acetic Acid **Thermo Fisher Scientific Catalog #**85183
3. Acetonitrile mass spectrometry grade **Thermo Scientific Catalog #**51101
4. Formic Acid **Thermo Scientific Catalog #**28905
5.

Equipment

Peptide Desalting Spin Columns	NAME
Thermo Scientific	BRAND
89851	SKU
https://www.thermofisher.com/order/catalog/product/89851	LINK

6.

Equipment

Low Retention Tubes and Tips	NAME
Brand	BRAND
0000000	SKU

Note

- The maximum volume for spin columns is 300 uL. If columns ever become unpacked, repeat the step that caused this by reloading the flowthrough and spinning at the recommended speed.

Reagents

Table 1: Reagent Preparation

Table Reagent Preparation	
Reagents	Amount
Priming buffer	100% Acetonitrile
Washing buffer	0.1% TFA in HPLC H_2O
Elution buffer	50% solution with 0.1% TFA in HPLC-grade water
Reconstitution buffer	5% solution with 0.1% Formic acid in HPLC-grade water

Protocol materials

- HPLC-grade water **Thermo Fisher Scientific Catalog #**51140
- Trifluoro-acetic Acid **Thermo Fisher Scientific Catalog #**85183
- Acetonitrile mass spectrometry grade **Thermo Scientific Catalog #**51101
- Formic Acid **Thermo Scientific Catalog #**28905
- Pierce Quantitative Colorimetric Peptide Assay **Thermo Fisher Scientific Catalog #**23275

Troubleshooting

Column Preparation

4m

1 **Column Preparation**

1.1 Take a Pierce peptide desalting spin column and remove the white tip (do not remove the screw cap of the tube). Place in a 2mL tube and spin column at 5000 x g for 00:01:00 . 1m

1.2 Add 300 µL of ***acetonitrile***. Spin at 5000 x g for 00:01:00 and discard flow-through. 1m

1.3 Repeat this step once 2m

Note

- Note that if columns ever become unpacked, repeat that step as the columns will not work properly if unpacked.

Sample Loading

2m

2 **Sample Loading**

2.1 Place the spin column in a new low-retention 2 mL tube labeled "flowthrough".

2.2 Load 300 µL of peptide sample into the tube and spin at 3000 x g for 00:01:00 . 1m

Note

- You can save the flow-through to ensure it does not contain any unbound peptides and that peptides are binding to the columns.

2.3 Based on the total sample volume, if:

2.3a) More than 300 µL of sample → place into a new 2 mL tube and load the remaining volume
2.3b)Less than 300 µL → reload the flow-through.

2.4 Spin the sample at 3000 x g for 00:01:00. Store "FT" at -80 °C for troubleshooting purpose. 1m

Wash 3m

3 **Wash Sample**

3.1 Place the spin column in a new low-retention 2mL-tube and load 300 µL of ***0.1% TFA in HPLC-grade H_2O***. Centrifuge at 3000 x g for 00:01:00. Discard wash flow-through. 1m

3.2 Repeat step 3.1 2 more times. 2m

Note

- Note that if columns ever become unpacked, repeat that step as the columns will not work properly if unpacked.

Sample Elution 1m

4 **Elute Samples**

4.1 Place the spin column in a new 2mL low-retention tube labeled with the sample name.

4.2 Load 300 µL of ***0.1% TFA, 50% acetonitrile in HPLC-grade H_2O*** . Spin at 3000 x g for 00:01:00 . 1m

Note

- Note that if columns ever become unpacked, repeat that step as the columns will not work properly if unpacked.

4.3 Transfer the spin column to another 2mL-low retention tube and repeat the step.

4.4 Pool the two elution samples from 4.2 and 4.3. These are the desalted peptides.

4.5 Store at -20 °C .

Lyophilization and Reconstitution

5 **Lyophilization**

5.1 To remove reagents incompatible with mass spectrometry place tubes in SpeedVac™ until completely dry.

5.2 Depending on the size of the peptide pellet, resuspend samples with 20 -75 µL of ***0.1% formic acid, 5% acetonitrile in HPLC-grade H_2O***.

5.3 Vortex until completely resuspended.

5.4 Peptide concentration can be measures using a spectrophotometer or using peptide concentration measurement kits such as:

Pierce Quantitative Colorimetric Peptide Assay **Thermo Fisher Scientific Catalog #**23275

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Protocol references

This protocol was adapted from Pierce™ Peptide Desalting Spin Columns User Guide, Catalog Numbers 89851 and 89852: **Thermo Fisher Pierce Peptide Desalting Spin Columns**