Aug 09, 2019

Peptide Desalting with a Vacuum Manifold

DOI
dx.doi.org/10.17504/protocols.io.3hegj3e

Rebecca E Hardman[1], Jeffrey A Lewis[2], Tara Stuecker[2]

[1]University of Arkansas - Fayetteville; [2]University of Arkansas

Lewis Lab

Rebecca E Hardman
University of Arkansas - Fayetteville

DOI: https://dx.doi.org/10.17504/protocols.io.3hegj3e

Protocol Citation: Rebecca E Hardman, Jeffrey A Lewis, Tara Stuecker 2019. Peptide Desalting with a Vacuum Manifold. **protocols.io https://dx.doi.org/10.17504/protocols.io.3hegj3e**

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

Created: May 29, 2019

Last Modified: August 09, 2019

Protocol Integer ID: 23814

Keywords: peptide, desalting, proteomics, peptide desalting, essential step for mass spectrometry workflow, mass spectrometry workflow, throughput desalting, vacuum manifold desalting

Abstract

Desalting of peptides is an essential step for mass spectrometry workflows. We detail the steps for medium-throughput desalting (10-20 samples) with Oasis HLB Cartidges.

Materials

MATERIALS

- Oasis 60ml Cartridges **Waters** **Catalog #**WAT094226
- Resprep® SPE Manifold **Restek** **Catalog #**26077
- Trifluoroacetic Acid Optima™ LC/MS Grade **Fisher Scientific** **Catalog #**A11650
- Acetonitrile ≥99.9% OmniSolv® LC-MS **VWR International (Avantor)** **Catalog #**EM-AX0156-6
- 18 guage needles **VWR International (Avantor)** **Catalog #**BD305195

Troubleshooting

Safety warnings

Trifluoroacetic acid (TFA) warnings from supplier: Causes severe burns by all exposure routes. Inhalation may cause central nervous system effects. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Hygroscopic. Use personal protective equipment. Use only under a chemical fume hood. Wash off immediately with plenty of water for at least 15 minutes. Immediate medical attention is required. Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Immediate medical attention is required. Move to fresh air. If breathing is difficult, give oxygen. If breathing is difficult, give oxygen. Do not use mouth-to-mouth resuscitation if victim ingested or inhaled the substance

Acetonitrile warnings from supplier: Highly flammable liquid and vapor. Harmful if swallowed, in contact with skin or if inhaled. Causes serious eye irritation. Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. Wear protective gloves/protective clothing/eye protection/face protection. In case of contact with eyes flush immediately with plenty of flowing water for 10 to 15 minutes holding eyelids apart and consult an ophthalmologist. Protect uninjured eye. Remove contact lenses, if present and easy to do. Continue rinsing. After contact with skin, wash immediately with plenty of water and soap. Remove contaminated, saturated clothing immediately. In case of skin reactions, consult a physician. If accidentally swallowed rinse the mouth with plenty of water (only if the person is conscious) and obtain immediate medical attention. Do NOT induce vomiting. Give nothing to eat or drink. IF exposed or concerned: Immediately call a POISON CENTER/doctor

Buffers

1 Prepare buffers:

a. 0.1% TFA – Make 15 ml per sample plus 1-2 ml extra.

i. For example: if you are desalting 10 samples, you would make 152 ml (1 5 ml x 10 samples = 150 ml plus 2 ml extra = 152 ml)

b. 70% Acetonitrile – Make 2 ml per sample plus 1-2 ml extra.

Preparing Vacuum Manifold

2 Set up vacuum manifold in chemical hood.

a. Attach feet to manifold.

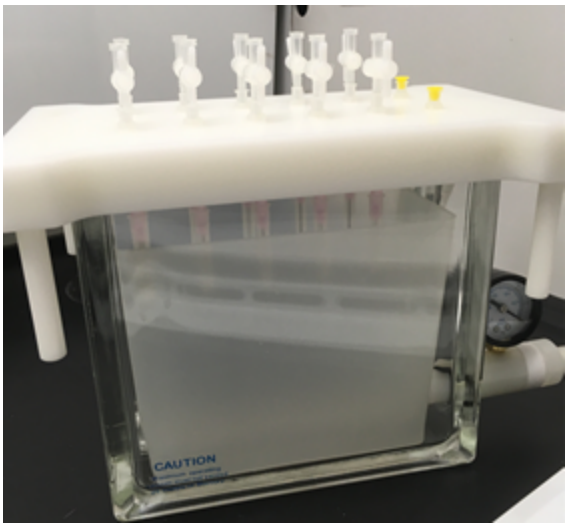
b. Prepare one port for each sample you will be desalting as follows:

i. Remove yellow stoppers – store them in a conical tube to make sure they do not get lost. Stoppers must remain in any unused ports to maintain the vacuum.

ii. Insert stopcocks. Twist them to make sure they are secure, then align them all so they are facing out.

iii. Make sure fasteners on under side of stopcocks (bottom side of manifold) are attached firmly, then attach 18 gauge needles (still sheathed) to each.

iv. Assemble manifold by placing square waste bucket into glass stand. Place manifold onto glass stand. MAKE SURE YOU (CAREFULLY) REMOVE SHEATHS FROM NEEDLES WHEN YOU PLACE MANIFOLD ON GLASS COLLECTION STAND.



v. Connect hose from manifold to vacuum pump.

vi. Make sure all stopcocks are in the closed position (up/down).

vii. Place one Oasis desalting column on each stopcock and label the columns with each sample name.

Preparing Columns

3 Activate columns with 3ml 100% Acetonitrile.

a. Pipette Acetonitrile onto columns and let it flow through by gravity (do not turn on vacuum pump).

Note

Be very careful not to let columns run dry during desalting process. For most steps, gravity will be enough for columns to drain. Only use the vacuum when necessary during the wash and elution steps. Watch the columns as they are draining. Once the liquid drains out, either immediately pipette the next step onto the column or close the stopcocks so it does not dry out.

4 Equilibrate column with 3 ml 0.1% TFA in water.

a. Pipette TFA onto columns and let it flow through by gravity (do not turn on vacuum pump). As soon as all liquid has passed into resin, close the stopcocks.

5 Remove plastic collection bucket from manifold and replace with collection rack containing 50 ml conical tubes for collecting flowthrough.

a. Label each conical tube with sample name.

Note

These samples are collected in case the peptides do not bind to the column. In that case, they can be recovered from the flowthrough.

Desalting

6 Pipette 3 ml of each sample into desalting columns and let them flow through by gravity.

a. Repeat with another 3 ml until all of sample has applied through the column.

Note

Be very careful not to mix up samples during this process and be careful not to let any columns run dry. If too many are ready at once, you can close the stopcocks.

7 Wash columns with 3ml 0.1% TFA, collecting this first wash into the same 50 ml conical tubes that have the flowthrough. When all of wash solution has passed through column,

close the stopcocks and wait until the wash step is complete for all samples

8 Pull out collection rack and replace it with the plastic collection bucket for the remaining washes, which will not be collected. Put lids on flowthrough/wash tubes and freeze at -80°C.

9 Repeat wash step 3 more times (3ml 0.1% TFA in water per wash) for a total of 4 washes.

Note

Mark a line on each tube as you add each wash to keep track of how many washes you have done. Some columns will flow faster than others, making it essential to record the the wash steps for each sample.

Note

If some columns are not draining by gravity, you can turn on the vacuum pump for this step. You generally do not need to dial up the vacuum--briefly turning on the pump is usually sufficient to restore flow. IF YOU DO USE THE VACUUM, MAKE SURE THE PRESSURE DOES NOT GO ABOVE THE MAX WRITTEN ON THE FRONT OF THE MANIFOLD.

10 Wash with 3ml ultrapure water using gravity.

Note

As with the previous wash, you can use the vacuum pump if necessary to restore flow.

11 Remove waste container from glass base and replace it with your elution tubes (15 ml conical tubes in collection rack—make sure you label your tubes with your sample names both on the sides of the tubes and the lids.)

12 Elute with 3 ml 70% Acetonitrile
a. Use gravity until there is no more visible liquid, and then pull a vacuum to get out the last remaining liquid.

13 Remove samples from collection rack and put lids on tubes.

a. If lyopholizing, freeze samples in liquid nitrogen.

Cleaning Manifold

14 To clean the vacuum manifold:

a. Dispose of acetontrile and TFA waste according to institutional guidelines.

b. Carefully remove and discard needles in sharps box.

c. Soak stopcocks in beaker with detergent to clean, then rinse well.

d. Rinse manifold with water, squirt soap solution through all ports and rinse thoroughly with purified water.

e. Do a final rinse with ethanol and dry on bench.