SPE peptide cleanup

This document describes a protocol for performing SPE cleanup of peptide samples prior to MS analysis. The approach described is based on the use of 96-well spin plates, but suggestions are given for adaptation to other types of media, such as SPE cartridges, StageTips, and TopTips.

Quick links

1. Peptide Sample Preparation

For all the solutions described below and throughout this protocol in general, you should do your best to use sterile glassware and reagents. As the goal here is to measure proteins, make an effort to minimize potential keratin contamination (e.g. wear a lab coat and don't touch your tubes with un-gloved hands).

1.1 Reagents and materials

- Benchtop centrifuge with holder for microcentrifuge tubes (multiple vendors)
- Clean water (Thermo Scientific, CAT#10977023)
- Strata-X microelution SPE plates (CAT# 8M-S100-4GA, Phenomenex)
  - Empore C18 disk (CAT#66884-U, Sigma)
  - Strata-X C18 10mg, 1mL tube (CAT#8B-S100-AAK, Phenomenex)
- Methanol, HPLC grade (CAT#610090040, Thermo Scientific)
- Water, HPLC grade (CAT#51140, Thermo Scientific)
- Trifluoroacetic acid, HPLC grade (CAT#85183, Thermo Scientific)
- Formic acid, HPLC grade (CAT#85178, Thermo Scientific)
- 1.5mL snap-lock tubes (CAT#05-402-25, Thermo Scientific)
- Benchtop centrifuge with plate-holder rotor (multiple vendors)
- AxyMats Sealing mat for 96-well microplates (VWR, CAT#14-222-024)
- 96-well PCR microplates (VWR, CAT#14-222-326)

1.2 Solution recipes

- Buffer A - methanol
- Buffer B - 0.1% trifluoroacetic acid (TFA) in water
- Rinse buffer - 0.1% formic acid, 4% methanol, in water
- Elution buffer - 0.1% formic acid in 60% methanol
- Sample Reconstitution Solution - 1% formic acid in water

1.3 Protocol

This protocol assumes you are using a 96-well spin plate. The plates listed in the materials have 2mg of material and as a general rule, we will assume a 1% binding capacity (e.g. 2mg of material can bind 20ug of peptide). In reality, it is likely higher than this, but it is better to err on the side of having more capacity than not enough. If you are using a StageTip, each plug will generally bind 2-4ug of peptide, so scale accordingly.

If you are using an SPE tube, 800uL of volume per step is generally a good starting point. If you are using a stage tip, 100uL will work well. If you are using a centrifuge for your StageTips, you may need to adjust your speeds to get a reasonable flow rate. The most important thing is that you don't want your bead bed to dry out prior to sample loading, especially with StageTips. Try to keep some volume above the top of the bead bed. The 96-well beads are water-wettable, so this is not as much of a concern.

1. Clean up your sample using a Strata-X SPE plate:
    1. Add 200uL of Buffer A to the SPE plate wells and centrifuge at 250g for 3-minutes. Empty the collection plate to the waste.
    2. Add 200uL of Buffer B to the SPE plate wells and centrifuge at 250g for 3-minutes. Empty the collection plate to the waste.
    3. Add 200uL of the peptide samples to the SPE plate wells and centrifuge at 250g for 3-minutes. Empty the collection plate to the waste.
    4. Repeat Step 3 to load the remainder of the sample.
    5. Add 200uL of Rinse buffer to the SPE plate wells and centrifuge at 250g for 3-minutes. Empty the collection plate to the waste.
    6. Add 200uL of Elution buffer to the SPE plate wells and centrifuge at 250g for 3-minutes.
    7. Transfer the elution from the collection plate to fresh 1.5mL tubes for each sample.
2. Concentrate the peptide sample by evaporation. You can use a SpeedVac or a Lyophilizer for this purpose.
3. After the sample is evaporated, add 20uL of Sample Reconstitution Solution to the tube, vortex, and then spin at 12,000g for 2-minutes.
4. Transfer 10uL of the reconstituted peptides to a 96-well plate with a silicone mat lid. Freeze the remainder of the peptide material at -80C.
5. Samples for MS analysis in the 96-well plate can be stored at this stage at -20C until analysis.