

**Arizona Proteomics Consortium Protocol**  
**08/12/10**

**C18 solid phase extraction clean up of peptides**

Equipment

Varian SPEC PT C18 pipette tips, catalog # A57203  
3 mL syringe  
1.5 mL eppendorf tubes  
pH paper  
speed vac

Reagents Use high quality reagents (we use HPLC grade)

Wetting solution: 100% MeOH  
Equilibration solution: 0.1% trifluoroacetic acid (TFA) in water  
Washing solution: 0.1% TFA  
Elution solution: 0.1% TFA/60% ACN

The SPEC pipette tips actually do not work with a pipettor; a 3 ml syringe needs to be used to generate enough suction.

1. Bring volume of solution to 100 ul. Check the pH of the sample, and, if necessary, add TFA until pH is around 3.
2. Wet resin with MeOH: before putting the tip onto the syringe, pull the plunger back 0.5 ml or so. Aspirate 50 ul MeOH, dispense it out and follow with an air push. Next aspirate and dispense 100 ul portions of equilibration solution 2 times.
3. Load sample, aspirating and dispensing 3 times (recycle sample).
4. Wash tip with 100 ul portions of wash solution 2 times. Discard washes.
5. Elute the peptides by aspirating and dispensing 100 ul of elution solution 3 times (recycle eluant).
6. Reduce volume in speed vac to 10 ul, store at -20°C.

Notes: with the exception of the MeOH air push, avoid aspirating air and do not let the resin dry out. Tip capacity is around 0.4 mg.