

SEP-PAK C18 PURIFICATION

Guidelines for sample preparation

(How to protect your samples from contamination with keratin)

- Clean your bench
 - Try to avoid contact of samples and solutions with dust, skin or hair
 - Wear gloves at all times
 - All reagents should be prepared fresh
 - Use ultra-pure water for all solutions
1. Attach a syringe to the Sep-Pak column (both should be attached to a vacuum manifold). Avoid air bubbles in column at any step during protocol.
 2. Equilibrate the column:
 - flush the column with **5 ml** of solution **B** or **Acetonitrile**
 - add **10 ml** of solution **A**. Add the peptide digest sample into the 10 ml of solution A and let it into the Sep-Pak column slowly.
 3. Wash the sample with **10 ml** of Solution **A**
 4. Elute with **2 x 600 µl** of solution **B** (collect your purified sample in a 2 ml tube)
 5. Dry down purified peptides completely in a speed-vac. Resuspend in Solution A (up to 20µl)

MATERIAL AND SOLUTIONS

Waters C18 Sep-Pack cartridges: Sep-Pak Plus™, WAT020515 (up to 1mg)
Light (up to ~100µg)
Thermo Solar

Solution A:

98% MilliQ-H₂O
2% CH₃CN
0.1% TFA (recommended) or FA

Solution B:

65% CH₃CN
35% MilliQ-H₂O
0.1% TFA (recommended) or FA
Use HPLC-grade Acetonitrile and FA, and MilliQ-H₂O.