STAGE (STop And Go Extraction) TIPS Desalting Procedure

Original Reference: Juri Rappsilber, Yasushi Ishihama and Matthias Mann, 2003. **St**op **A**nd **G**o **E**xtraction **Tip**s for Matrix-Assisted Laser Desorption/Ionization, Nanoelectrospray, and LC/MS Sample Pretreatment in Proteomics. Anal. Chem. 75, 663-670.

Notes about sample composition:

a) Acidify samples (pH 4 or less)

b) Ensure that organic (acetonitrile, methanol) concentration is at or below 5 – 10%

c) Reversed-phase material does not remove some detergents (e.g., SDS) and other hydrophobic contaminants, therefore perform detergent removal before C18 extraction.

d) Adjust the glycerol composition of samples (if present) to 5 or 10% in order to reduce viscosity of the solvent

Materials for Stage Tip assembly:

1. Empore reversed-phase extraction disks from 3M (SDB-XC reversed-phase material, 3M product number 2240/2340)
2. 17 or 18 gauge blunt ended syringe needle
3. 200 μl pipette tips
4. 0.3 or 0.5 um ID (PEEK or fused silica) tubing
5. 1.5 mL microfuge tubes

Stage Tip assembly (P200 pipette tip with Empore C18 disk cores): Place Empore disk flat on a clean hard surface, for instance a glass microscope slide. Press the (17 or 18 gauge) blunt ended syringe needle into the Empore disk to core out a piece of the filter material. Press a second core into the syringe needle for extra loading capacity. Place the needle into a 200 μL pipette tip and push the cored disk pieces into the pipette tip with PEEK or fused silica tubing. Gently pack the material into the end of the pipette tip; a gap of several millimeters should be visible between the disk and the end of the tip. Do not overpack or underpack. Estimate of binding capacity of a '2-punch' Stage Tip is approximately 15 ug.

Stage Tip/Tube assembly: Cut a cap from a 1.5 mL Eppendorf tube; bore a hole into the center of the cap; snap the cap onto a new 1.5 mL Eppendorf tube; place a pipette tip fitted with Empore disk cores into the hole in the cap. The tip of the pipette tip should be about 1 cm from the bottom of the tube. Alter the size of the hole in the lid if necessary. Prepare 1 cap/tip/tube assembly per sample.

DESALTING PROCEDURE (revised from original method)

Soln 1: Wash solvent: 98:2:0.1%, water:acetonitrile:trifluoroacetic acid (TFA)

Soln 2: Wetting solvent: 80:20:0.1%, acetonitrile:water:trifluoroacetic acid (TFA)

Soln 3: Elution solvent: 40:60:0.1%, acetonitrile:water:trifluoroacetic acid (TFA)

Prepare fresh solvents weekly; do not pipette neat TFA with plastic pipette tips, use glass syringe.

Follow protocol below for a 2-punch Stage Tip; reduce solvent amounts by 50% if for 1-punch Stage tip.

1. Reconstitute samples in 60 µl wash solvent (**Soln 1** '98:2:0.1%'), vortex 45 sec; centrifuge 3000 x g for 1 min. CHECK pH (pipette 0.5 µl onto pH strip). Ensure pH is ≤3. (Adjust with 10% aqueous TFA if necessary, in 0.5 µl increments, for example.)

2. Pipette 60 µl wetting solvent (**Soln 2** '80:20:0.1%') onto Stage Tip/Tube assembly. Centrifuge 450 x g for 2 minutes.

3. Pipette 60 µl wash solvent (**Soln 1** '98:2:0.1%') onto the Stage Tip/Tube assembly. Centrifuge 450 x g for 2 minutes.

4. Discard liquid in bottom of Eppendorf tube, replace cap/StageTip.

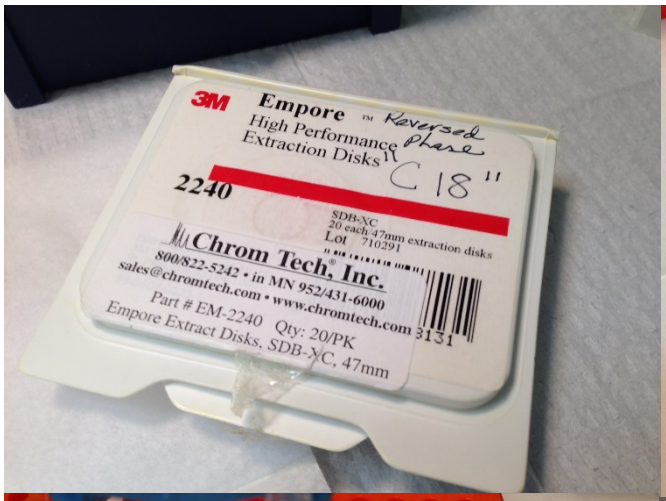
5. Pipette samples into Stage Tip. Centrifuge 450 x g for 2 minutes. Ensure solvent is washed through Stage Tip; increase centrifuge time if necessary; do not over-centrifuge.

6. Wash C18 material: Pipette 60 µl wash solvent (**Soln 1** '98:2:0.1%') onto the Stage Tip/Tube assembly. Centrifuge 450 x g for 2 minutes. **Repeat**.

7. Place cap/Stage Tip assembly onto a new 1.5 mL Eppendorf tube; label tube w/'**ST C18', sample name and your initials.**

8. Elute peptides from C18 material: Pipette 60 µl elution solvent (**Soln 3** '40:60:0.1%') onto the Stage Tip/Tube assembly. Centrifuge 450 x g for 2 minutes.

9. Speed vacuum desalted peptide mixture to dryness.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