

## **STAGE (STop And Go Extraction) TIPS Clean up Procedure:**

### **“MCX-type” Protocol for Detergent and Salt Removal**

*Original Reference (for C18 clean up)* Juri Rappsilber, Yasushi Ishihama and Matthias Mann, 2003. **Stop And Go Extraction Tips** for Matrix-Assisted Laser Desorption/Ionization, Nanoelectrospray, and LC/MS Sample Pretreatment in Proteomics. *Anal. Chem.* 75, 663-670.

#### **Notes about sample composition:**

- a) Reconstitute samples in 60  $\mu$ l sample reconstitution solvent (0.2% formic acid (FA) in ultrapure water); ensure  $\text{pH} \leq 3$
- b) Ensure that organic (acetonitrile, methanol) concentration is at or below 5 – 10%
- c) 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 SDB-RPS extraction disks from 3M (mixed mode strong cation exchange and reversed-phase material, 3M product number EM-2241; purchase from Fisher or VWR)
2. 17 or 18 gauge blunt ended syringe needle
3. 200  $\mu$ l pipette tips
4. 0.3 or 0.5  $\mu$ m ID (PEEK or fused silica) tubing
5. 1.5 mL microfuge tubes

**Stage Tip assembly (P200 pipette tip with Empore SDB-RPS disk cores):** Place Empore disk (or membrane) 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  $\mu$ L 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 per core is 2-4  $\mu$ g

**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.

**Prepare solvents;** *Prepare fresh solvents bi-weekly; do not pipette neat FA with plastic pipette tips, use glass syringe.*

- **Sample reconstitution solvent:** 0.2% formic acid (FA) in ultrapure water
- **Conditioning solvent A:** acetonitrile
- **Conditioning solvent B:** ultrapure water
- **Wash solvent A:** 95:5:0.2%, water : acetonitrile : formic acid (FA)
- **Wash solvent B:** acetonitrile
- **Elution solvent:** 40:55:5%, acetonitrile : water : ammonium hydroxide

**PROCEDURE** *Follow protocol below for a 2-core Stage Tip; reduce solvent amounts by 50% if a 1-core Stage tip is use. Inspect Stage Tip after each centrifugation step, increase time (or force, in small increments) if solvent did not pass through extraction disk/membrane*

1. Reconstitute samples in 60  $\mu$ l sample reconstitution solvent, vortex 45 sec; centrifuge 3000 x g for 1 min; ensure pH  $\leq$  3.
2. **Condition:** Pipette 60  $\mu$ l acetonitrile onto membrane. Centrifuge 450 x g for 2 min.
3. **Condition:** Pipette 60  $\mu$ l ultrapure water onto membrane. Centrifuge 450 x g for 2 min.
4. Load samples into Stage Tip. Centrifuge 450 x g for 2 min. Ensure solvent is washed through Stage Tip; increase centrifuge time or force if necessary; do not over-centrifuge.
5. **Wash 1 and 2:** Pipette 60  $\mu$ l wash solvent A (95:5:0.2%, water : acetonitrile : formic acid) onto membrane. Centrifuge 450 x g for 2 minutes. **Repeat.**
6. **Wash 3:** Pipette 60  $\mu$ l acetonitrile onto membrane. Centrifuge 450 x g for 2 minutes.
7. Place cap/Stage Tip assembly onto a new 1.5 mL Eppendorf tube; label tube as 'eluate.'
8. **Elute:** Pipette 60  $\mu$ l elution solvent (40:55:5%, acetonitrile : water : ammonium hydroxide) onto membrane. Centrifuge 450 x g for 2 minutes.
9. Speed vacuum peptide mixture to dryness. Check pH before RP LC-MS analysis.

