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. **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) Reconstitute samples in 60 ul sample reconstitution solvent (0.2% formic acid (FA) in ultrapure water); ensure pH ≤ 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 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 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 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 per core is 2-4 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.

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:** 60:35:5%, 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 μl sample reconstitution solvent, vortex 45 sec; centrifuge 3000 x g for 1 min; ensure pH ≤ 3.
2. **Condition:** Pipette 60 μl acetonitrile onto membrane. Centrifuge 450 x g for 2 min.
3. **Condition:** Pipette 60 μl 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 μl 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 μl 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 μl elution solvent (60:35:5%, 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 anlaysis.


Solid Phase Extraction Disk
Empore ™
2241 - Styrenedivinylbenzene - Reversed Phase Sulfonate (SDB-RPS)
47 mm
20 Each
Lot # 760174D
MCX
Bioanalytical Technologies
3M Company
3M Center, Building 270-2A-08
St. Paul, MN 55144-1000
1-888-509-5330
www.3m.com/empore
Made in USA
3M
34-7046-3778-3


SCI
SCIENTIFIC COMMODITIES
BLUNT TIP PIPETTING NEEDLES
#BB829-2-18 18 GAUGE 2" LONG
PACK OF 3 NEEDLES
PHONE#: 800-331-7724
www.scicominc.com