

## Operating Instructions

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### **UltraMicroSpin™ (2-40µL elution volume, 5-50 µg max. capacity) and MicroSpin™ Columns (5-100µL elution volume, 10-100 µg max. capacity)**

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#### **Directions for Strong Cation Exchange Chromatography (SUM HIL-SCX & SEM HIL-SCX):**

- Slide the adapter collar onto the spin column and place it in a 2ml micro centrifuge tube.
- Conditioning the column:** Pipette 100 µl of wetting solvent (e.g., 100% methanol) into the column and centrifuge it for 1 min. at about 110x g (@ ~800 rpm with an Eppendorf micro centrifuge). Flush with at least 2 bed volumes (50 or 100µl, respectively) of 100% water before elution with any salt solution to prevent salt precipitation. Condition the cartridge with a strong buffer for at least one hour prior to its initial use (add 100µl of conditioning buffer, spin for 10 sec. and let stand in the tube). A convenient solution to use is 0.2 M monosodium phosphate + 0.3 M sodium acetate (the pH will be between 3.0 and 6.5). Flush with 50µL, 100% water.
- Equilibrate** the column with 100µl of 5mM phosphate (or ammonium formate), pH 3.0 and centrifuge it for 1 min. at about 110x g (@200 rpm with an Eppendorf micro centrifuge). Repeat two more times. Remove the collecting tube and blot dry any moisture on the exterior of the column.
- Processing the sample:** Add your 2-100µl of sample (in 5mM, pH 3.0 buffer) to the column and place it in a new 2ml centrifuge tube. Spin the tube 1 min. at 110x g. Peptides and proteins will be retained, while detergents and non polar solutes will elute in the liquid in the collecting tube if some organic solvent is present in the buffer. Discard this liquid. Add an additional 50 µl of equilibration solvent and repeat the spin to wash out any trace impurities or un-retained detergent. Repeat once again if necessary.
- Releasing the sample:** Add 2-50µl of water to the tube, containing 5mM phosphate + 0.1 - 0.8M NaCl (or even ammonium formate, although elution would be broader under these conditions). Spin as above. Peptides and proteins will be in the liquid in the collection tube. If a sample is especially basic, repeat this step with increasing amounts of salt to elute all of the sample.

**NOTE: These spin columns of PolySULFOETHYL Aspartamide™ will retain cationic solutes such as peptides, protein digests, or simple organic amines.**

- Since total binding capacity is on the order of 0.05-0.5 mg depending on column size. There will be a considerable Donnan exclusion effect present. To prevent exclusion from the column put sample in 5-15 mM of salt or buffer. Additionally, the gradient will be much more concave than that expected. Thorough equilibration is necessary prior to loading to ensure retention. Conditioning and equilibration of cartridges can be done in advance, then stored in the refrigerator until needed.
- Columns can be reused by washing three times with two bed volumes (50µL, 100µL or 500µL, respectively) of 800mM NaCl (aq.) or 80% MeCN, and then washing three times with two bed volumes of the 5mM conditioning solvent.
- Peptides are retained by the positive charge of at least the n-terminal amine and eluted by a combination of total charge, charge distribution and hydrophobicity. If your peptide does not stick to the column, be sure it is in a small amount of buffer, or decrease the concentration of organic in the A & B solvents to 10 or 5%. (Organic solvent concentration is empirically determined). Detergents, and non-polar solutes will not be retained if some organic solvent is used in the mobile phase.
- Use of 0.1% TFA or high concentrations of formic acid in the mobile phase is not recommended. The conditioning process is reversed by exposing the column to pure organic solvents. Use pH 3 for retention of neutral to slightly acidic peptides. Use of a higher pH may be considered for basic hydrophobic peptides.
- Sample composition: Important:** The sample and the conditioning solvent should contain comparable amounts of acetonitrile (e.g., 5+%), and salt concentrations should be 5-15 mM. Otherwise, polar solutes such as peptides and proteins might not be retained. Dilute the sample if necessary to decrease the salt concentration.

### **MacroSpin™ Columns (50-150µL elution volume, 50-500 µg capacity)**

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#### **Directions for Strong Cation Exchange Chromatography (p/n: SMM HIL-SCX):**

- Conditioning the column:** Pipette 500µl of wetting solvent (e.g., 100% methanol) into the column and centrifuge it for 1 min. at about 110x g (@ ~800 rpm with an Eppendorf micro centrifuge). Flush with at least 2 bed volumes (500µl) of 100% water before elution with any salt solution to prevent salt precipitation. Condition the cartridge with a strong buffer for at least one hour prior to its initial use (add 500µl of conditioning buffer, spin for 10 sec. and let stand in the tube). A convenient solution to use is 0.2 M monosodium phosphate + 0.3 M sodium acetate (the pH will be between 3.0 and 6.5).
- Equilibrate** the column with 500µl of 5mM phosphate, pH 3.0 and centrifuge it for 1 min. at about 110x g (@200 rpm with an Eppendorf micro centrifuge). Repeat two more times. Remove the collecting tube and blot dry any moisture on the exterior of the column.
- Processing the sample:** Add your 50-150µl of sample (in 5mM, pH 3.0 buffer) to the column and place it in a new 2ml centrifuge tube. Spin the tube 1 min. at 110x g. Peptides and proteins will be retained, while detergents and non polar solutes will elute in the liquid in the collecting tube if some organic solvent is present in the buffer. Discard this liquid. Add an additional 100 µl of equilibration solvent and repeat the spin to wash out any trace impurities or un-retained detergent. Repeat once again if necessary.
- Releasing the sample:** Add 50-150µl of water to the tube, containing 5mM phosphate + 0.1 - 0.8M NaCl (or even ammonium formate, although elution would be broader under these conditions). Spin as above. Peptides and proteins will be in the liquid in the collection tube. If a sample is especially basic, repeat this step with increasing amounts of salt to elute all of the sample.

### **96-Well Spin and 96-Well MACROSpin Plates (50-100µL elution volume, 10-100µg max. capacity and 50-150µL elution volume, 50-500 µg max. capacity, respectively).**

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#### **Directions Strong Cation Exchange Chromatography (SCX): (p/n: SNS HIL-SCX & SNS HIL-SCX-L):**

- Tap the column gently to ensure that the dry column material is settled at the bottom of the columns and condition as above. Foil is for sealing purposes only. All 96 wells do not need to be opened at the same time. Remove foil from as many rows as desired for your application. Foil should be cut with a razor or other sharp blade.
- Place the 96-Well Spin Column into a collection plate and pipette 200µL of organic solvent (400µL for the MACROSpin Plates) into all opened wells and centrifuge the plate for 1 minute on the collection plate, at 110x g to wet the SCX phase. Then repeat with 95-100% water to remove all organic. Condition with high salt buffer as above, remove that buffer with water as above, and equilibrate with 25% organic containing low amounts of volatile buffer with the appropriate volumes as for the MicroSpin and MACROSpin, respectively.
- You can reuse the emptied collection plate for sample loading. Blot dry any liquid on the exterior of the column. Add your 50-100µL sample (50-150µL for the MACROSpin Plates) to the top of a well. Be careful to ensure that the sample is placed in the center of the well.
- Place the column in a new collection plate when the appropriate elution solvent is added (i.e. higher concentrations of salt), spin the plate for 1 minute at 110x g, using the volume recommendations above. After centrifugation, the purified sample will be in the collecting tube and will be ready for further use. It may be necessary to repeat this step to elute all of the sample.