

## Operating Instructions

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These spin columns of PolyHYDROXYETHYL Aspartamide™ will retain polar solutes such as peptides, proteins, and polar metabolites. Salts, detergents, and non polar solutes will not be retained. This permits the removal of nonvolatile components from samples prior to mass spectrometry, removal of toxic substances prior to bioassay, and preliminary fractionation of a mixture by polarity differences.

### **UltraMicroSpin™ (2-40µL elution volume, 3-30µg max. capacity) and MicroSpin™ Columns (5-100µL elution volume, 6-60µg max. capacity)**

#### **Directions Hydrophilic Interaction Chromatography (HILIC): (p/n: SUM HIL & SEM HIL):**

- 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 100% water.
- Equilibrate** the column with 100µl of 70-85% acetonitrile containing 15-25mM ammonium formate (or some other suitable buffer), pH 6.0 and centrifuge it for 1 min. at about 110x g (@ ~800 rpm with an Eppendorf micro centrifuge). Repeat once. Remove the collecting tube and blot dry any moisture on the exterior of the column.
- Processing the sample:** Dilute your sample with acetonitrile (to 70-85% final conc., depending on your molecule), and add 2-100 µl of this (in 15-25mM, pH 6.0 buffer) to the column placed in a clean 2ml centrifuge tube. Spin the tube 1 min. at 110x g. Peptides and proteins will be retained, while non-polar detergents and non-polar solutes will elute in the liquid in the collecting tube. Discard this liquid. Add an additional 50 µl of equilibration solvent and repeat the spin to wash out any traces detergent. Repeat once, if necessary.
- Releasing the sample:** Add 2-50µl of water to the tube, containing 15mM formic acid or some other electrolyte. Spin as above. Peptides and proteins will be in the liquid in the collection tube. If a sample is especially polar, repeat this step with increasing amounts of salt to elute all of the sample (see Note D, below).

#### **NOTES:**

- Columns can be reused by washing three times with two bed volumes (50µL, 100µL or 500µL, respectively) of 15-25 mM formic acid (aq.) and then washing three times with two bed volumes of conditioning solvent.
- Sample composition: *Important:* The sample and the conditioning solvent should contain comparable amounts of acetonitrile (e.g., 80+%), and salt concentrations should be < 40 mM. Otherwise, polar solutes such as peptides and proteins might not be retained. Dilute the sample with water, if necessary to decrease the salt concentration, prior to dilution with organic.
- Instead of stepping directly from 85% to 0% acetonitrile, it is possible to perform washes with intermediate levels of acetonitrile, such as 60% and/or 40%. This sometimes permits the resolution of a mixture into several fractions containing solutes with different degrees of polarity.
- Basic groups are the most polar, followed by phosphate groups. Asn- and Ser- are the most polar neutral amino acids. For details on Hydrophilic Interaction Chromatography, see Alpert, J. Chromatogr. 499 (1990) 177-196.

### **MacroSpin™ Columns (50-150µL elution volume, 30-300 µg capacity)**

#### **Directions Hydrophilic Interaction Chromatography (HILIC): (p/n: SMM HIL):**

- Conditioning the column:** Pipette 400 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 (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 400µl 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 100% water.
- Equilibrate** the column with 400µl of 85% acetonitrile, 15-25mM ammonium formate (or some other suitable buffer), pH 6.0 and centrifuge it for 1 min. at about 110x g (@ ~800 rpm with an Eppendorf micro centrifuge). Repeat once. Remove the collecting tube and blot dry any moisture on the exterior of the column.
- Processing the sample:** Dilute your sample with acetonitrile (to 70-85% final conc., depending on your molecule), and add 50-150µl of this (in 15-25mM, pH 6.0 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 non-polar detergents and non-polar solutes will elute in the liquid in the collecting tube. Discard this liquid. Add an additional 250µl of equilibration solvent and repeat the spin to wash out any traces detergent or other non-polar impurities. Repeat once, if necessary.
- Releasing the sample:** Add 50-150 µl of water to the tube, containing 15-25mM formic acid or some other electrolyte. Spin as above. Peptides and proteins will be in the liquid in the collection tube. If a sample is especially polar, repeat this step with increasing amounts of salt to elute all of the sample (see Note D, above).

### **96-Well Spin and 96-Well MACROSpin Plates (5-100µL elution volume, 6-60µg max. capacity and 50-150µL elution volume, 30-300µg max. capacity, respectively).**

#### **Directions Hydrophilic Interaction Chromatography (HILIC): (p/n: SNS HIL & SNS HIL-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 HILIC 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 high 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 water), 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. If a sample is especially polar see **Notes** above. It may be necessary to repeat this step to elute all of the sample.