

## Operating Instructions

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### **UltraMicroSpin™ (2-100µL elution volume, 3-30µg capacity) and MicroSpin™ Columns (5-200µL elution volume, 6-60µg capacity)**

#### **Directions for Weak Anion Exchange Chromatography (p/n: SUM HIL-DE & SEM HIL-DE):**

These spin columns of PolyWAX LP™ will retain anionic solutes such as peptides or protein digests. This permits the fractionation of digests by negative charge prior to RPC desalting and hydrophobic fractionation for mass spectrometry. Capacities are modest (30 - 300µg).

- 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 (50µL 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).
- **Equilibrate** the column with 100µl of 15mM phosphate, pH 6.0 and centrifuge it for 1 min. at about 110x g (@ ~800 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 2-200µl of sample (in 15mM , 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 if some organic solvent is present in the buffer. (However, SDS will be retained.) Discard this liquid. Add an additional 25-50µl 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 15mM phosphate + 0.1 - 0.5M NaCl or some other salt. Spin as above. Peptides and proteins will be in the liquid in the collection tube. If a sample is especially acidic repeat this step with increasing amounts of salt to elute all of the sample. . Alternatively, one can use 5 - 25µl of 0.1% ammonium hydroxide to desorb all retained species. If SDS is present, consider using the HILIC cartridges (p/n: SUM HIL or SEM HIL) to remove it.

#### NOTE:

- Peptides are retained by their negative charge. If your peptide does not stick to the column, be sure it is in a small amount of buffer.
- 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 15mM conditioning solvent.
- **Sample composition: Important:** The sample and the conditioning solvent should contain comparable amounts of acetonitrile (e.g., 5+%), and salt concentrations should be 15 mM. Otherwise, polar solutes such as peptides and proteins might not be retained. Dilute the sample if necessary to decrease the salt concentration.
- Instead of stepping directly from 0% to 100% NaCl, it is possible to perform washes with intermediate levels of NaCl, such as 10%, 20%, 30%, etc.. This permits the fractionation of a mixture into several fractions containing solutes with different numbers of negative charges.
- Conditioning and equilibration of cartridges can be done in advance, then stored in the refrigerator until needed.

### **MacroSpin™ Columns (50-450µL elution volume, 30-300µg capacity)**

#### **Directions for Weak Anion Exchange Chromatography (SMM HIL-DEAE):**

- **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 600µl of 15mM phosphate, pH 6.0 and centrifuge it for 1 min. at about 110x g (@ ~800 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 50-450µl of sample (in 15mM , 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 if some organic solvent is present in the buffer. (However, SDS will be retained.) 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 50-250µl of water to the tube, containing 15mM phosphate + 0.1 - 0.5M NaCl or some other salt. Spin as above. Peptides and proteins will be in the liquid in the collection tube. If a sample is especially acidic repeat this step with increasing amounts of salt to elute all of the sample. Alternatively, one can use 50-150µl of 0.1% ammonium hydroxide to desorb all retained species.