UltraMicroSpin™ ™ (2-25µL elution volume, 5-50 µg max. capacity) and *MicroSpin™ Columns (5-50µL elution volume, 10-100 µg max. 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 phospho-peptides or acidic glycans. This also permits the fractionation of digests in an e-HILIC mode for mass spectrometry (See the HILIC instructions for conditions). Capacities are modest, but thorough equilibration is necessary to achieve maximum binding.

- 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). Flush with 50µL, 100% water.
- **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 2-100µl 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, and then stored in the refrigerator in 25% organic until needed.

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

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

- **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 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 50-150µl 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 50-150µl 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.

96-Well Spin and 96-Well MACROSpin Plates (10-50µL elution volume, 10-100 µg max. capacity and 40-150µL elution volume, 50-500 µg max. capacity, respectively).

Directions Weak Anion Exchange Chromatography (WAX): (p/n: SNS HIL-DE & SNS HIL-DE-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 WAX 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 using the volumes appropriate for the MicroSpin and MACROSpin, as above, 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.