UltraMicroSpin™ *(2-25µL elution volume, 3-30µg capacity)* **and** ***MicroSpin™ Columns*** *(5-50µL elution volume, 6-60µg capacity)*

Directions for Weak Anion Exchange Chromatography ***(p/n: SUM WAX & SEM WAX)****:*

These spin columns of Sepharose DEAE™ will retain anionic (acidic pI) solutes. 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 into the column and centrifuge it for 1 min. at about 110x g (@ ~800 rpm with an Eppendorf micro centrifuge). 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).
- **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. Proteins will be retained. Discard this liquid. Add an additional 25-50µl of equilibration solvent and repeat the spin to wash out any trace impurities, which do not bind. Repeat once again if necessary.
- **Releasing the sample**: Add 2-50µl of 15mM phosphate + 0.1 - 0.3M NaCl, some other salt; pH 3.0, 15mM acid (to uncharged the carboxyls of the protein), or 0.1% ammonium hydroxide (to uncharged the amines of the column). Spin as above. 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.

NOTE:

- Proteins are retained by their negative charges. If your protein does not stick to the column, be sure it contains a small concentration of buffer.
- Columns can be reused by washing three times with two bed volumes (50µL, 100µL or 500µL, respectively) of 800mM NaCl (aq.), 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 buffer. Concentrations should be *ca.* 15 mM. Otherwise, negative solutes, such as peptides, 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 equal volume elutions with intermediate levels of NaCl, such as 10%, 20%, 30%, etc.. This permits the fractionation of a mixture into several fractions containing solutes with an increasing number of negative charges.
- Conditioning and equilibration of cartridges can be done in advance, then stored in the refrigerator until needed, if 0.1% sodium azide is used in the storage buffer. To remove, spin three times with two bed volumes of the 15mM equilibration solvent.

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

Directions for Weak Anion Exchange Chromatography ***(SMM WAX)****:*

- **Conditioning the column**: Pipette 400µl of wetting solvent into the column and centrifuge it for 1 min. at about 110x g (@ ~800 rpm with an Eppendorf micro centrifuge). Condition the cartridge with a strong buffer for at least one hour prior to its initial use (i.e., add 400µl of conditioning buffer, spin for 10 sec. and let stand in the tube).
- **Equilibrate** the column with 400µl 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. Proteins will be retained. Discard this liquid. Add an additional 150µl of equilibration solvent and repeat the spin to wash out any trace impurities, which do not bind. Repeat once again if necessary.
- **Releasing the sample**: Add 50-150µl of 15mM phosphate + 0.1 - 0.3M NaCl, some other salt; pH 3.0, 15mM acid (to uncharged the carboxyls of the protein), or 0.1% ammonium hydroxide (to uncharged the amines of the column). Spin as above. 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.

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 for Weak Anion Exchange Chromatography ***(SNS WAX & SNS WAX-L)****:*

- Tap the column gently to ensure that the dry column material is settled at the bottom of the columns.
- 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 onto a collection plate.
- Place 200µL of buffer in all open wells (400µL for MACROSpin Plates). Wait 15 minutes for hydration. Follow the instructions above, based on your column size, for conditioning & equilibration. Centrifuge the plate for 3 minutes in a 96-Well collection plate at 110x g to remove the excess buffer.
- Remove the 96-Well Spin Column from the collection plate. Blot dry any moisture on the exterior of the column. Add 10-60µL sample (MicroSpin Plate) or 40-125µl (MACROSpin Plate) of sample to the top of a well. Be careful to ensure that the sample is placed in the center of the well. Wash to column as many times as is required for your specific application.
- **Releasing the sample**: Place the column on a new collection plate. Add 50-150µl of 15mM phosphate + 0.1 - 0.3M NaCl, some other salt; pH 3.0, 15mM acid (to uncharged the carboxyls of the protein), or 0.1% ammonium hydroxide (to uncharged the amines of the column). Spin the plate for 3 minutes at 110x g. After centrifugation, the purified sample will be in the collecting tube and will be ready for further use. If a sample is especially acidic repeat this step with increasing amounts of salt to elute all of the sample.

NOTE: Each plate is designed for a one-time application only, and it is recommended that plates should not be re-used since the quality of results could be affected.