MicroSpin™ Columns (5-40µL elution volume, 60 µg capacity)

Directions for SDS Detergent Removal Chromatography (PUMSCSDS1203):

These spin columns will retain anionic detergents and other strongly anionic solutes such as phospho-peptides from protein digests. They should be used when one does not want to utilize HILIC (high organic) conditions for the removal of detergents. They permit the retention of SDS (below 45% MeCN) from digests prior to RPC desalting or hydrophobic fractionation for mass spectrometry. Capacities are modest (50 -100µg).

- 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 15mM phosphate, pH 3.0 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 5-25µl of sample (in 15mM , pH 3.0 buffer) to the column and place it in a new 2ml centrifuge tube. Spin the tube 1 min. at 110x g. Most peptides and proteins will not be retained, while SDS and other anionic detergents will be retained in less than 45% MeCN. Add an additional 50 µl of equilibration solvent and repeat the spin to wash out any traces sample. Repeat once again if necessary.
- **Releasing the SDS**: Add 25-50µl of 90% MeCN. Spin as above. If a sample is especially acidic repeat this step with increasing amounts of salt to elute all of the sample. . Alternatively, one can use 10 - 100µl of 0.1% ammonium hydroxide to desorb all retained species.

NOTE:

- Some peptides, especially phosphopeptides, might be retained by their negative charge. If your peptide does stick to the column, try removing SDS at low pH (,pH 3.0) instead using a 15mM of a buffer. Alternatively, consider using HILIC cartridges or HILIC conditions on these cartridges, to remove the SDS and retain the peptides.
- Columns can be reused by washing three times with two bed volumes (50µL, 100µL or 500µL, respectively) of 800mM NaCl (aq.) **then** 90% 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 might not be retained. Dilute the sample if necessary to decrease the salt concentration.
- Conditioning and equilibration of cartridges can be done in advance, then stored in the refrigerator until needed.

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

Directions for SDS Detergent Removal Chromatography (PMASCSDS1203):

- **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 15mM phosphate, pH 3.0 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 3.0 buffer) to the column and place it in a new 2ml centrifuge tube. Spin the tube 1 min. at 110x g. Most peptides and proteins will not be retained, while SDS and other anionic detergents will be retained in less than 45% MeCN. Add an additional 250µl of equilibration solvent and repeat the spin to wash out any traces sample. Repeat once again if necessary.
- **Releasing the SDS**: Add 50-150µl of 90% MeCN. Spin as above. 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.