BioPureSPN™ MACRO HIL-SCX Used as IEX Columns & 96-Well Plates

(50-200µl elution volume, 35-350 µg (HILIC) 02-1.0 mg (IEX) maximum capacity)

When used in an ion exchange mode, these spin columns of PolySULFOETHYL A™ SCX will retain peptides and proteins with as little as a single positive charge and will remove non-ionic and acidic detergents in the presence of 30% organic solvent. Salts, and polar, negatively charged solutes, like DNA, will not be retained. Use for preliminary fractionation by positive charge differ-ences for MS samples.

Directions: ***(p/n: HMM HIL-SCX, HMM HIL-SCX.20, HNS HIL-SCX-L)****: Cut Off The Outlet Tab with Side-Cut Pliers or a Box Cutter against an elevated support surface. Loosen The Cap when spinning.*

- **Conditioning the column**: Pipette 400µl of conditioning solvent (e.g., 100% acetonitrile or MeOH) into the column and centrifuge for 1 min. at about 55 x g (@ ~400 rpm with an Eppendorf micro centrifuge). Flush with 1 tube volume (~400µl ea.) of 100% water. Flush with at least 2 bed volumes (200µL) 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). Spin out remaining strong buffer and flush with 200µL, 100% water.
- **Equilibrating the column for IEX**: Pipette 200µl of 5mM phosphate (or 25mM ammonium formate), pH 3.0 and centrifuge it for 1 min. at about 30 x g (@~200 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**: (***Note:*** *When using fixed-angle rotors, place a mark on the upper side of the column. Place column in centrifuge with the mark facing outward in all subsequent centrifugation steps. Improper orientation will result in reduced recovery efficiency.*) Load your 50-200µl of sample (in 5mM , pH 3.0 buffer) to the column and place it in a new 2ml centrifuge tube. Spin the tube 1 min. at 110x g. Pep- tides and proteins will be retained, while detergents and non polar solutes will elute in the liquid in the collecting tube (if some organic solvent is present in the buffer). Discard (or save) this liquid. Rinse with 50-100µl of loading or equilibration buffer to wash out any traces of impurities from your sample of interest.
- **Releasing the sample**: Add 50-200µl of 5mM phosphate + 0.1 - 0.8M NaCl (or 500mM ammonium formate or some other volatile electrolyte) to completely wet the frit, preferably containing 5-20% ACN to reduce hydrophobic association. Spin as above. Peptides and proteins will be in the liquid in the collection tube. If a sample is especially non-polar it may be necessary to repeat this step to elute all of the sample (See SDS note below). If especially basic, repeat this step with increasing amounts of salt to elute all of the sample.

NOTES:

- These spin columns of PolySULFOETHYL A™ will retain cationic solutes such as peptides, protein digests, or simple organic amines.

BioPureSPN™ MACRO HIL-SCX Used as HILIC (ERLIC) Columns & 96-Well Plates

(50-200µl elution volume, 35-350 µg (HILIC) 0.2-1.0 mg (IEX) maximum capacity)

When used in a HILIC (ERLIC) mode, these columns of PolySULFOETHYL A™ SCX will retain soluble peptides and will remove non-ionic and acidic detergents during the load in > 50% organ-ic solvent. More non-polar, and negatively charged solutes will be less retained. Use for pre-liminary fractionation by polarity differences for MS samples.

*Directions: **(p/n: HMM HIL-SCX, HMM HIL-SCX.20, HNS HIL-SCX-L)**: Cut Off The Outlet Tab with Side-Cut Pliers or a Box Cutter against an elevated support surface. Loosen The Cap when spinning.*

- **Conditioning the column**: Pipette 400µL of conditioning solvent (e.g., 100% acetonitrile or MeOH) into the column and centrifuge for 1 min. at about 55 x g (@ ~400 rpm with an Eppendorf micro centrifuge).
- **Equilibrating the column for HILIC**: Flush with 1 tube volume of 85% ACN, 5mM - 20mM ammonium formate (or acetate) at a pH appropriate for the separation (e.g. pH 3 to enhance fractionation by neutral and positive charges, or pH 6 to partially repel negative charged species and retain neutral components). Centrifuge for 1 min. at 30 x g (@ ~200 rpm on an Eppendorf micro centrifuge). Repeat twice. Remove the collecting tube and blot dry any moisture on the exterior of the column.
- **Processing the sample**: (***Note:*** *When using fixed-angle rotors, place a mark on the upper side of the column. Place column in centrifuge with the mark facing outward in all subsequent centrifugation steps. Improper orientation will result in reduced recovery efficiency.*) Load 50-200µL of sample (in the same buffer at an appropriate pH) to the column and place it in a new 2mL centrifuge tube. Spin the tube 1 min. at 110x g. Polar analytes will be retained, while detergents and non-polar solutes will elute in the liquid in the collecting tube. Discard (or save) this liquid. Rinse with 50-100µL of loading or equi libration buffer to wash out any traces of impurities from your sample of interest.
- **Releasing the sample**: Add 50-200µL of a higher concentration of ammonium formate or some other volatile electrolyte in 40% ACN to completely wet the frit. Spin as above. Polar analytes will be in the collection tube. If a sample is especially polar it may be necessary to repeat this step to elute all of the sample. If especially basic, repeat this step with increasing amounts of volatile salt to elute all of the sample.

NOTES:

- For a discussion of the ERLIC technique see the ERLIC WAX Dropbox on our web site: http://www.nestgrp.com

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