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

(50-200µL elution volume, 17-170 µg (HILIC) maximum capacity)

When used in a HILIC (ERLIC) mode, these columns of PolyHYDROXYETHYL A™ will retain soluble peptides and will remove non-ionic and cationic detergents during the load in > 50% organic solvent. More non-polar solutes will be retained more. Use for preliminary fractionation by polarity differences for MS samples.

Directions: ***(p/n: HEM HIL, HEM HIL.20, HNS HIL-M)****: 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 200µL of conditioning solvent (e.g., 100% acetonitrile or MeOH) into the column and centrifuge until "dry" at about 55x g (@ ~400 rpm on 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 6.5 to enhance fractionation by neutral and negative charges, or pH 3 to retain components a neutral column surface.). Centrifuge for until "dry" at ~55x g (@ ~400 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. Also too high a spin speed will decrease the binding and/or elution effectiveness.***) Load 25-100µL 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 until "dry" at ~55x 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 equilibration buffer to wash out any traces of impurities from your sample of interest.
- **Releasing the sample**: Add 50-200µL of 40% ACN, 20mM - 100mM ammonium formate or some other volatile electrolyte to completely wet the frit. Spin as above. Peptides and proteins will be in the liquid in the collection tube. If a sample is especially ionic it may be necessary to repeat this step 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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