

BioPureSPN™ HIL MACRO Used as HILIC (ERLIC) Columns & 96-Well Plates
(50-200µL elution volume, 35-350 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: **HMM HIL, HMM HIL.20, HNS HIL-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 6.5 to enhance fractionation by neutral and negative charges, or pH 3 to retain components a neutral column surface.). 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 55 x 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 50-200µL 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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