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

(50-200µL elution volume, 35-350 µg (HILIC) 0.2-1 mg (IEX) maximum capacity)

When used in an ion exchange mode, these spin columns of PolyWAX LP™ WAX will retain peptides and proteins with multiple negative charges and will remove non-ionic and basic detergents inthe presence of 30% organic solvent. Polar and negatively charged solutes will be preferentially retained. Use for preliminary fractionation by negative and neutral charge differences for MS samples.

Directions: ***(p/n: HMM HIL-WAX, HMM HIL-WAX.20, HNS HIL-WAX-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 least2 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 200µL 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 6.5 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 col- umn. Place column in centrifuge with the mark facing outward in all subsequent centrifugation steps. Improper ori- entation will result in reduced recovery efficiency.*) Load your 50-200µL of sample (in 5mM , pH 6.5 buf- fer) to the column and place it in a new 2ml centrifuge tube. Spin the tube 1 min. at 110x g. Peptides 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 - 0.4M 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 willbe in the liquid in the collection tube. If a sample is especially ionic it may be necessary to re- peat this step to elute all of the sample. If especially acidic (poly-phosphorylated peptides), re- peat this step with increasing amounts of salt to elute all of the sample at pH 2.0.

NOTES:

- These spin columns of PolyWAX LP™ will retain anionic solutes such as phosphopeptides or proteins.

BioPureSPN™ MACRO HIL-WAX 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 PolyWAX LP™ WAX will retain soluble peptides and will remove non-ionic and cationic detergents during the load in > 50% organic solvent. More non-polar, and positively charged solutes will be less retained than anionic ones. Use for preliminary fractionation by polarity differences for MS samples.

Directions: ***(p/n: HMM HIL-WAX, HMM HIL-WAX.20, HNS HIL-WAX-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. at110x 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 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. If especially acidic (poly-phosphorylated peptides), repeat this step with increasing amounts of salt to elute all of the sample at pH 2.0.

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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