BioPureSPN™ Mini HIL-PSA Used as IEX Columns & 96-Well Plates

(20-100µL elution volume, 7-70 µg (HILIC) 40-200 µg (IEX) maximum capacity)

When used in an ion exchange mode, these primary-secondary amine spin columns, PSA™ ERLIC, will retain peptides and proteins with multiple negative charges and will remove non-ionic and basic detergents in the presence of 30% organic solvent. Use for preliminary fractionation by negative and neutral charge differences for MS samples with volatile buffers.

Directions: ***(p/n: HUM HIL-PSA, HUM HIL-PSA.20, HNS HIL-PSA)****: 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 25x g (@ ~200 rpm with an Eppen- dorf micro centrifuge). Flush with 1 tube volume (~400µL ea.) of 100% water. Flush with at least 2 bed volumes (40µL) of 100% water before elution with any salt solution to prevent salt precipi- tation. 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 solu- tion 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 50µL, 100% water.
- **Equilibrating the column for IEX**: Pipette 100µL of 5mM phosphate (or 25mM ammonium formate), pH 6.5 and centrifuge it until "dry" at about 25x g (@ ~200 rpm with an Eppendorf mi- cro 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. Also too high a spin speed will decrease the binding and/or elution effectiveness.***) Load your 10-40µL of sample (in 5mM , pH 6.5 buffer) to the column and place it in a new 2ml centrifuge tube. Spin the tube until "dry" at 25x 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 20-40µL of loading or equilibration buffer to wash out any traces of impuri- ties from your sample of interest.
- **Releasing the sample**: Add 20-100µL of 5mM phosphate + 0.1 - 0.4M NaCl (or 500mM am- monium formate or some other volatile electrolyte) to completely wet the frit, preferably contain- ing 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 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.

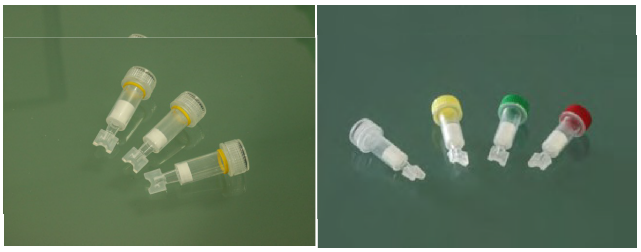
NOTES:

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

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

(20-100µL elution volume, 7-70 µg (HILIC) 40-200 µg (IEX) maximum capacity)

When used in a HILIC (ERLIC) mode, these primary-secondary amine spin columns, PSA™ ERLIC, 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: HUM HIL-PSA, HUM HIL-PSA.20, HNS HIL-PSA)****: 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 25x g (@ ~200 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 until “dry” at 25x 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. Also too high a spin speed will decrease the binding and/or elution effectiveness.***) Load 10-40µL 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 25x 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 20-40µL of loading or equi- libration buffer to wash out any traces of impurities from your sample of interest.
- **Releasing the sample**: Add 20-100µL 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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The Nest Group, Inc.
17 Hayward St., Ipswich, MA 01938-2041
TEL# 508-481-6223
http://www.nestgrp.com
FAX# 508-485-5736
E-mail: sales@nestgrp.biz