UltraMicroSpin™ (2-40µL elution volume, 3-30µg max. capacity)* and *MicroSpin™ Columns (5-100µL elution volume, 6-60µg max. capacity)

*Directions for ERLIC SPE WAX Phosphopeptide Enrichment **(p/n: SUM HIL-DE & SEM HIL-DE)**:*

These spin columns of PolyWAX LP™ will retain anionic solutes such as phosphopeptides or acidic glycans. This permits their enrichment from digests in an ERLIC mode for mass spectrometry. Capacities are modest, but thorough equilibration is necessary to achieve maximum binding.

- Slide the adapter collar onto the spin column and place it in a 2ml micro centrifuge tube.
- **Conditioning the column**: Pipette 100 µl of wetting solvent (e.g., 100% methanol) into the column and centrifuge it for 1 min. at about 110x g (@ ~800 rpm with an Eppendorf micro centrifuge). Flush with at least 2 bed volumes (50µL or 100µl, respectively) 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). Flush with 50µL, 100% water.
- **Equilibrate** the column with 70% ACN containing 20mM Na-MePO3, pH 2.0 (titrate methyl-phosphonic acid with NaOH prior to adding the ACN). Centrifuge for 1 min. at about 110x g (@ ~800 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**: Dilute your sample with acetonitrile (to 70-85% final conc.), and add 2-100µl of this (i.e. 70% ACN, 20mM, pH 2.0 Na-MePO3 buffer) to the column placed in a clean 2mL centrifuge tube. Spin the tube 1 min. at 110x g. Phosphopeptides will be retained, while lipids, detergents (even SDS at 70% acetonitrile), and unmodified acidic peptides will elute during the load into the collecting tube. Discard this liquid. Add an additional 50µl of equilibration solvent and repeat the spin to wash out any traces of non-phosphorylated peptides. Discard this liquid.
- **Releasing the sample**: Add 2-50µl of 10% ACN with 20mM, pH 2.0 buffer to the tube to elute most mono-phosphorylated peptides. Spin as above. Mono-phosphorylated peptides will be in the liquid in the collection tube, while poly-phosphylated peptides will remain on the column. An elution with 50mM NaH2PO4 or KH2PO4 buffer, pH 2.0 in 10% ACN will elute any remaining mono-phosphorylated peptides and almost all di-phosphorylated peptides. A final 50µl elution with 300mM TEAP (or NaH2PO4 or KH2PO4) will elute poly-phosphorylated peptides. To remove these non-volatile salts, consider using TARGA C18 cartridges (p/n: SUM SS18R or SEM SS18R) to bind peptides and to wash away any salt with 100% water. However, first evaporate off all of the acetonitrile to assure maximal retention on the TARGA C18 in 100% water.
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NOTE: For more details on ERLIC, see: A.J. Alpert. Electrostatic Repulsion Hydrophilic Interaction Chromatography for Isocratic Separation of Charged Solutes and Selective Isolation of Phosphopeptides. *Anal. Chem.* **80** (2008) 62-76; or ASMS 2008 Poster: Isolation of Tryptic Phosphopeptides by ERLIC.

- Peptides are retained by their negative charge. If your peptide does not stick to the column, be sure it is in a small amount of buffer.
- **Sample composition**: *Important*: The sample and the conditioning solvent should contain comparable amounts of acetonitrile (e.g., 5+%), and salt concentrations should be 20 mM. Dilute the sample if necessary to decrease the salt concentration.
- Conditioning and equilibration of cartridges can be done in advance, and then stored in the refrigerator in 25-50% organic with a soluble buffer until needed.

MacroSpin™ Columns (50-150µL elution volume, 30-300µg capacity)

*Directions for ERLIC SPE WAX Phosphopeptide Enrichment **(SMM HIL-DE)**:*

- **Conditioning the column**: Pipette 400 µl of wetting solvent (e.g., 100% methanol) into the column and centrifuge it for 1 min. at about 110x g (@ ~800 rpm with an Eppendorf micro centrifuge). Flush with at least 2 bed volumes (500µl) 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 400µl 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). Flush with 100% water.
- **Equilibrate** the column with 400µl of 70% ACN containing 20mM Na-MePO3, pH 2.0 (see above) and centrifuge for 1 min. at about 110x g (@ ~800 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**: Dilute your sample with acetonitrile (to 70-85% final conc.), and add 50-150µl of this (i.e. 70% ACN with 20mM, pH 2.0 Na-MePO3 buffer) to the column placed in a clean 2ml centrifuge tube. Spin the tube 1 min. at 110x g. Phosphopeptides will be retained, while lipids, detergents (even SDS at 70% acetonitrile), and unmodified acidic peptides will elute into the collecting tube. Discard this liquid. Add an additional 150µl of equilibration solvent and repeat the spin to wash out any traces of non-phosphorylated peptides. Discard this liquid.
- **Releasing the sample**: Add 50-150µl of 10% ACN with 20mM, pH 2.0 buffer to the tube to elute mono-phosphorylated peptides. Spin as above. Mono-phosphorylated peptides will be in the liquid in the collection tube, while poly-phosphylated peptides will remain on the column. An elution with 50mM NaH2PO4 or KH2PO4 buffer, pH 2.0 in 10% ACN will elute any remaining mono-phosphorylated peptides and almost all di-phosphorylated peptides. A final 150µl elution with 300mM TEAP (or NaH2PO4 or KH2PO4) will elute poly-phosphorylated peptides. To remove these non-volatile salts, consider using TARGA C18 cartridges (p/n: SMM SS18R) to bind peptides and to wash away any salt with 100% water. However, first evaporate off all of the acetonitrile to assure maximal retention on the TARGA C18 in 100% water.
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96-Well Spin and 96-Well MACROSpin Plates (50-100µL elution volume, 6-60µg max. capacity and 50-150µL elution volume, 30-300µg max. capacity, respectively).

Directions ERLIC SPE WAX Phosphopeptide Enrichment: (p/n: SNS HIL-DE & SNS HIL-DE-L):

- Tap the column gently to ensure that the dry column material is settled at the bottom of the columns and condition as above. Foil is for sealing purposes only. All 96 wells do not need to be opened at the same time. Remove foil from as many rows as desired for your application. Foil should be cut with a razor or other sharp blade.
- Place the 96-Well Spin Column into a collection plate and pipette 200µL of organic solvent (400µL for the MACROSpin Plates) into all opened wells and centrifuge the plate for 1 minute on the collection plate, at 110x g to wet the WAX phase. Then repeat with 95-100% water to remove all organic. Condition with high salt buffer as above, remove that buffer with water as above, and equilibrate with 70% organic containing 20mM pH 2.0 buffer using the volumes appropriate for the MicroSpin and MACROSpin, as above, respectively.
- You can reuse the emptied collection plate for sample loading. Blot dry any liquid on the exterior of the column. Add your 50-100µL sample (50-150µL for the MACROSpin Plates), as outlined above, to the top of a well. Be careful to ensure that the sample is placed in the center of the well.
- Place the column in a new collection plate when the appropriate elution solvent is added, spin the plate for 1 minute at 110x g until dry, using the volume and eluting solvent recommendations above. After centrifugation, the purified sample will be in the collecting tube and will be

ready for further use. It may be necessary to repeat this step to elute all of the sample or to desalt with TARGA C18 plates, SNS SS18R and SNS SS18R-L, after evaporating off all of the acetonitrile.