BioPureSPN™ Midi Desalting Columns & 96-Well Plates

(25-100µl elution volume, 17-170 µg maximum capacity)

These spin columns of PROTO™ 300 C4 (yellow cap) or C18 (green cap) will retain non-polar solutes such as peptides, proteins, and detergents. Salts, and polar solutes, like DNA, will not be retained permitting the removal of salts and SDS from MS samples or preliminary fractionation by hydrophobic differences. Use of 0.1% TFA will increase the binding of peptides and proteins.

Directions: ***(p/n: HEM S18V, HEM S04V)****: Snap Off The Outlet Tab & Loosen The Cap*

- **Conditioning the column**: Pipette 200µl of conditioning solvent (e.g., 100% acetonitrile or MeOH) into the column and centrifuge it for 1 min. at about 110 x g (@ ~800 rpm with an Eppendorf micro centrifuge). Flush with 1 tube volume (~200µl ea.) of 98% - 100% water.
- **Equilibrating the column**: Pipette 200µl of equilibrating solvent (e.g., 2% acetonitrile or MeOH) into the column and centrifuge it for 1 min. Repeat once. 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.*) Add 25-100µl of sample to the column and place it in a new 2ml centrifuge tube. Spin the tube 1 min. at 110 x g. Peptides, proteins and detergents will be retained, while, salts, and polar solutes, like DNA and alkylating reagents will elute. Discard this liquid unless these are the molecules you are after. Rinse with 10-40µl of loading or equilibration buffer to wash out any traces of salts from your sample of interest.
- **Releasing the sample**: Add 25-100µl of 80% MeCN or MeOH to completely wet the frit, preferably containing 25mM formic acid or some other volatile electrolyte. Spin as above. Peptides and proteins will be in the liquid in the collection tube. If a sample is especially non-polar it may be necessary to repeat this step to elute all of the sample (See SDS note below). If especially polar, then bind and equilibrate in 100% water containing 0.1% TFA or use the more retentive (for hydrophilic compounds), water wettable, **BioPureSPN™ TARGA®** C18 (p/n HEM S18R).

NOTES:

- Columns can be reused by washing three times with two bed volumes (50µL ea.) of 100% MeCN, MeOH or *n*-PrOH containing 25 mM formic acid (aq.) Drying will not affect performance. Cap during storage. Wash with two tube volumes (200µL ea.) of loading or equilibration buffer before re-use.

- **Sample composition**: *Important*: The sample and the equilibration buffer should contain comparable amounts of acetonitrile (e.g., 0 - 5%). Otherwise, polar solutes such as peptides and proteins might not be retained. Including 0.1% TFA increases binding capacity for peptide capture. Decrease the organic solvent concentration of the sample if yields are low from flow through.

- **SDS elution**: SDS elutes at 60-65% MeCN from C18 columns. If your analyte is more polar, elute it with 50% MeCN to prevent the SDS from moving off the column. Use of too much rinse or elution volume will promote isocratic elution, so limit these volumes to one void volume (50µL). Alternatively, use of a HILIC SPE will retain the peptides and elute the SDS during the loading, allowing elution of peptides in 100% water.

The Nest Group, Inc. TEL# 508-481-6223 FAX# 508-485-5736
45 Valley Road, Southboro, MA 01772-1323 http://www.nestgrp.com E-mail: sales@nestgrp.biz

BioPureSPN™ Midi TARGA® FastEq Desalting Columns & 96-Well Plates

(25–100µl elution volume, 17-170 µg maximum capacity)

These **Fast Equilibrating** spin columns, containing TARGA C18 (red cap), equilibrate 10x faster than other C18's, assuring sample retention. They can be loaded in 100% water or buffer solutions to retain weakly polar solutes such as peptides and proteins. Salts will not be retained, permitting their removal from MS samples. Alkylating reagents can be eluted during the rinse step with 2% MeCN. Use of 0.1% TFA, which increases the binding of peptides, is not necessary with these water wettable columns, although it can be used.

Directions: ***(p/n: HEM S18R)****: Snap Off The Outlet Tab & Loosen The Cap*

- **Conditioning the column**: Pipette 200µl of conditioning solvent (e.g., 100% acetonitrile or MeOH) into the column and centrifuge it for 1 min. at about 110 x g (@ ~800 rpm with an Eppendorf micro centrifuge). Flush with 1 tube volume (~200µl ea.) of 100% water.
- **Equilibrating the column**: Pipette 200µl of equilibrating solvent (e.g., 0% - 2% acetonitrile or MeOH) into the column and centrifuge it for 1 min. 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.*) Add 25-100µl of sample to the column and place it in a new 2ml centrifuge tube. Spin the tube 1 min. at 110x g. Peptides, proteins and detergents will be retained, while, salts, and polar solutes will elute in the liquid in the collecting tube. Discard this liquid unless these are the molecules you are after. Rinse with 50-100µl of loading or equilibration buffer and repeat the spin to wash out any traces of salts from sample of interest. Or rinse with 2-5% MeCN to remove weakly hydrophobic alkylating reagents.
- **Releasing the sample**: Add 25-100µl of 80% MeCN or MeOH to completely wet the frit, preferably containing 25mM formic acid or some other volatile electrolyte. Spin as above. Peptides and proteins will be in the liquid in the collection tube. If a sample is especially non-polar it may be necessary to repeat this step to elute all of the sample. If especially polar, then bind and equilibrate in 100% water containing 0.1% TFA

NOTES:

- Columns can be reused by washing three times with two bed volumes (50µL ea.) of 100% MeCN, MeOH or *n*-PrOH containing 25 mM formic acid (aq.) Drying will not affect performance. Cap during storage. Wash with two tube volumes (200µL ea.) of loading or equilibration buffer before re-use.

- **Sample composition**: *Important*: The sample and the equilibration buffer should contain comparable amounts of acetonitrile (e.g., 0 - 5%). Otherwise, polar solutes such as peptides and proteins might not be retained. Including 0.1% TFA increases binding capacity for peptide capture. Decrease the organic solvent concentration of the sample if yields are low from flow through.

- **SDS elution**: SDS elutes at 60-65% MeCN from C18 columns. If your analyte is more polar, elute it with 50% MeCN to prevent the SDS from moving off the column. Use of too much rinse or elution volume will promote isocratic elution, so limit these volumes to one void volume (50µL). Alternatively, use of a HILIC SPE will retain the peptides and elute the SDS during the loading, allowing elution of peptides in 100% water.

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