UltraMicroSpin™ RPsm ***(2-25µL elution volume, 3-30 µg max. capacity)*** **and** ***MicroSpin™ RPsm Columns*** ***(5-50µL elution volume, 6-60 µg max. capacity)***

Directions for TARGA Reversed Phase ***(RPsm):(p/n: SUM SS18R & SEM SS18R)****:*

These spin columns of water wettable TARGA C18 will retain polar & non-polar solutes such as carbohydrates, nucleotides, polar peptides as well as metabolites and pharmaceutical compounds. Salts will not be retained. This permits the removal of salt from samples prior to mass spectrometry, removal of toxic substances prior to bioassay, and preliminary fractionation of a mixture by hydrophobic differences. Use of 0.1% TFA will increase the binding of peptides and proteins although this phase does not require TFA for sharp desorption.

- Slide the adapter collar onto the spin column and place it in a 2ml micro centrifuge tube.
- **Conditioning the column**: Pipette 100µl of conditioning solvent (e.g., 100% acetonitrile or MeOH) into the column and centrifuge it for 1 min. at about 110x g (@ ~800 rpm with an Eppendorf micro centrifuge). Flush with 2 bed volumes (50 or 100µl, respectively) of 100% water. Remove the collecting tube and blot dry any moisture on the exterior of the column.
- **Processing the sample**: Add your 2-100 µl 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 metabolites will be retained, while, salts will elute in the liquid in the collecting tube. Discard this liquid unless these are the molecules you are after. Add an additional 25 or 50µl of loading or equilibration buffer and repeat the spin to wash out any traces of salts from sample of interest. Repeat once again if necessary.
- **Releasing the sample**: Add 2-50µl of 80% MeCN or MeOH to the tube, 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 (See Note A below), it may be necessary to repeat this step to elute all of the sample. If especially polar, then bind and equilibrate in 100% water.

NOTES:

- Columns can be reused by washing three times with two column volumes (100µL, 200µL or 400µL, respectively) of 100% MeCN, MeOH or *n*-PrOH containing 25 mM formic acid (aq.) and then washing with two bed volumes of loading or equilibration buffer (e.g., 100% water).
- **Sample composition**: *Important*: The sample and the equilibration buffer should contain comparable amounts of acetonitrile, otherwise polar solutes such as nucleotides might not be retained. Including 0.1% TFA increases binding capacity for zwitterionic analyte capture. Decrease the organic solvent concentration of the sample if yields are low.

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

Directions for TARGA Reversed Phase ***(RPsm): (p/n: SMM SS18R)****:*

- **Conditioning the column**: Pipette 400µl 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 400µl of 100% water. Remove the collecting tube and blot dry any moisture on the exterior of the column.
- **Processing the sample**: Add your 50-400µl 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, will elute in the liquid in the collecting tube. Discard this liquid unless these are the molecules you are after. Add an additional 50-150µl of loading or equilibration buffer and repeat the spin to wash out any traces of salts from sample of interest. Repeat once again if necessary.
- **Releasing the sample**: Add 50 - 150µL of 80% MeCN or MeOH to the tube, preferably containing 25mM formic acid or some other volatile electrolyte. Spin as above. Non-polar analytes will be in the liquid in the collection tube. If a sample is especially non-polar (See Note A above), it may be necessary to repeat this step to elute all of the sample. If especially polar, then bind and equilibrate in 100% water.

96-Well MicroSpin and 96-Well MACROSpin RPsm Plates ***(10-60µL elution volume, 10-100 µg max. capacity and 40-120µL elution volume, 40-400 µg max. capacity, respectively).***

Directions for TARGA Reversed Phase ***(RPsm): (p/n: SNS SS18R, or SNS SS18R-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 100µL of organic solvent (200µL for the MACROSpin Plates) into all opened wells and centrifuge the plate for 1 minute in the collection plate at 110x g to wet the TARGA C18 phase then repeat with 100% water to equilibrate.
- You can reuse the emptied collection plate for sample loading. Blot dry any liquid on the exterior of the column. Add your 30-60µL sample (60-120µL for the MACROSpin Plates) to the top of a well. Be careful to ensure that the sample is placed in the center of the well. Having 0.1% TFA in the sample can facilitate binding, of zwitterions, but it isn't necessary.
- Place the column in a new collection plate when the appropriate elution solvent is added (i.e. higher concentrations of MeCN or organic solvent). Spin the plate for 1 minute at 110x g. After centrifugation, the purified sample will be in the collecting tube and will be ready for further use. If a sample is especially non-polar (See Note A above), it may be necessary to repeat this step to elute all of the sample.