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

*Directions for Activated Charcoal:**(p/n: SUM SC00- & SEM SC00-)**:*

These spin columns of Activated Charcoal will retain polar solutes such as glycans. Salts will not be retained. This permits the removal of salts from samples prior to mass spectrometry. Use of 0.1.0% TFA will increase the elution of charged glycans.

- 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). Then flush (successively) with 2 bed volumes (50 or 100µl, respectively) of 1N NaOH, 30% HOAc, 100% ACN, and then 5% ACN: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. Glycans and proteins 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 (5% ACN) and repeat the spin to wash out any traces of salts. Repeat once again if necessary.
- **Releasing the sample**: Add 5-50µl of 30-50% MeCN or MeOH to the tube. Spin as above. Glycans will be in the liquid in the collection tube. If a sample is especially charged, it may be necessary add 0.1% TFA or to repeat this step to elute all of the sample.

NOTES:

- **Sample composition**: *Important*: The sample and the equilibration buffer should contain comparable amounts of acetonitrile (e.g., 0 - 5%).
- For more detailed binding and release conditions, see: **http://www.freepatentsonline.com/6376663.html .**

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

*Directions for Activated Charcoal: **(p/n: SMM SC00-)**:*

- **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 110x g (@ ~800 rpm with an Eppendorf micro centrifuge). Then flush (successively) with 2 bed volumes (500µl) of 1N NaOH, 30% HOAc, 100% ACN, and then 5% ACN:water. Remove the collecting tube and blot dry any moisture on the exterior of the column.
- **Processing the sample**: Add your 50 - 150µl of sample to the column and place it in a new 2ml centrifuge tube. Spin the tube 1 min. at 110 x g. Glycans and proteins 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 (5% ACN) and repeat the spin to wash out any traces of salts. Repeat once again if necessary.
- **Releasing the sample**: Add 50-150µl of 30-50% MeCN or MeOH to the tube. Spin as above. Glycans will be in the liquid in the collection tube. If a sample is especially charged, it may be necessary add 0.1% TFA or to repeat this step to elute all of the sample.

96-Well Spin and 96-Well MACROSpin Activated Charcoal Plates (5-100µL elution volume, 6-60µg max. capacity and 50-150µL elution volume, 30-300µg max. capacity, respectively).

*Directions for Activated Charcoal: **(p/n: SNS SC00-)***

- 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 into all opened wells and centrifuge the plate for 1 minute in the collection plate at 110x g to wet the Active Charcoal phase then follow instructions for the MicroSpin tubes, SEM SC00-, above to Condition the plate and Process the sample.
- 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 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 and elute with 10-50µL of the appropriate elution solvent (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 charged, it may be necessary add 0.1% TFA or to repeat this step to elute all of the sample.