

## Operating Instructions

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These spin columns of unbonded silica will retain polar solutes. Use of TLC plates will facilitate the methods development steps. Using non-polar organic solvents permits fractionation by polarity differences.

### **UltraMicroSpin™ (2-40µL elution volume, 5-50 µg max. capacity) and MicroSpin™ Columns (5-100µL elution volume, 10-100 µg max. capacity)**

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*Directions Normal Phase Chromatography: (p/n: SUM SS10 & SEM SS10):*

- Slide the adapter collar onto the spin column and place it in a 2ml micro centrifuge tube.
- **(optional) Conditioning the column:** Pipette 100 µl of wetting solvent (e.g., 100% hexane) into the column and centrifuge it for 1 min. at about 110x g (@ ~800 rpm with an Eppendorf micro centrifuge), unless you want to have even stronger retention of your small molecules onto the silica. In that case, simply load the sample onto the dry column.
- **(optional) Equilibrate** the column with 100µl of solvent and centrifuge it for 1 min. at about 110x g (@ ~800 rpm with an Eppendorf micro centrifuge). Repeat once. Remove the collecting tube and blot dry any liquid on the exterior of the column.
- **Processing the sample:** Dilute your sample with the equilibration solvent and add 2-200 µl to the column and place it in a new 2ml centrifuge tube. Spin the tube 1 min. at 110x g. More polar molecules will be retained, while non-polar solutes will elute in the liquid in the collecting tube. Discard this liquid. Add an additional 50 µl of equilibration solvent and repeat the spin to wash out any traces impurities. Repeat once, if necessary.
- **Releasing the sample:** Add 2-50µl of methanol or other polar solvent mixtures to the tube. Spin as above. Polar molecules will be in the liquid in the collection tube. If a sample is especially polar, repeat this step with increasing amounts of polar solvent mixtures to elute all of the sample (see Note D, below).
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### **MacroSpin™ Columns (50-150µL elution volume, 50-500 µg capacity)**

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*Directions Normal Phase Chromatography: (p/n: SMM SS10):*

- **(optional) Conditioning the column:** Pipette 500 µl of wetting solvent (e.g., 100% hexane) into the column and centrifuge it for 1 min. at about 110x g (@ ~800 rpm with an Eppendorf micro centrifuge), unless you want to have even stronger retention of your small molecules onto the silica. In that case, simply load the sample onto the dry column.
- **(optional) Equilibrate** the column with 500µl of solvent and centrifuge it for 1 min. at about 110x g (@ ~800 rpm with an Eppendorf micro centrifuge). Repeat once. Remove the collecting tube and blot dry any liquid on the exterior of the column.
- **Processing the sample:** Dilute your sample with the equilibration solvent and add 50-450 µl to the column and place it in a new 2ml centrifuge tube. Spin the tube 1 min. at 110x g. More polar molecules will be retained, while non-polar solutes will elute in the liquid in the collecting tube. Discard this liquid. Add an additional 50 µl of conditioning solvent and repeat the spin to wash out any traces impurities. Repeat once, if necessary.
- **Releasing the sample:** Add 50-250µl of methanol or other polar solvent mixtures to the tube. Spin as above. Polar molecules will be in the liquid in the collection tube. If a sample is especially polar, repeat this step with increasing amounts of polar solvent mixtures to elute all of the sample (see Note D, below).
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### **96-Well Spin Plate Kit (5-100µL elution volume, 10-100 µg capacity)**

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*Directions Normal Phase Chromatography: (p/n: SNS SS10):*

- **(optional) Conditioning the column:** Pipette 100 µl of wetting solvent (e.g., 100% hexane) into the column and centrifuge it for 1 min. at about 110x g (@ ~800 rpm with an Eppendorf micro centrifuge), unless you want to have even stronger retention of your small molecules onto the silica. In that case, simply load the sample onto the dry column.
- **(optional) Equilibrate** the column with 100µl of solvent and centrifuge it for 1 min. at about 110x g (@ ~800 rpm with an Eppendorf micro centrifuge). Repeat once. Remove the collecting tube and blot dry any liquid on the exterior of the column.
- **Processing the sample:** Dilute your sample with the equilibration solvent and add 2-200 µl to the column and place it in a new 2ml centrifuge tube. Spin the tube 1 min. at 110x g. More polar molecules will be retained, while non-polar solutes will elute in the liquid in the collecting tube. Discard this liquid. Add an additional 50 µl of equilibration solvent and repeat the spin to wash out any traces impurities. Repeat once, if necessary.
- **Releasing the sample:** Add 2-50µl of methanol or other polar solvent mixtures to the tube. Spin as above. Polar molecules will be in the liquid in the collection tube. If a sample is especially polar, repeat this step with increasing amounts of polar solvent mixtures to elute all of the sample (see Note D, below).

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