OBSOLETE

SuproTipTM General Instructions:

Directions:

- 1. To begin wash with several Tip volumes of 50:50, MeCN: PrOH. Repeat 2-3 times. This will reduce the low MW ions in ESI-MS and will activate the media.
- 2. Wash with 100% water. Be sure to remove all of the activation solvent, as it will promote elution of sample during the binding step.
- 3. Condition SuproTip media with two pipette volumes of binding solvent appropriate for the chemistry used (100% water for RPC, 70-95% MeCN or MeOH for HILIC; H₂O with 5mM buffer and 1.8M NH₄SO₄ for HIC; H₂O with 5mM HOAc buffer for IEX; Hexane for silica normal phase, EDTA for IMAC.). Pull liquid into tip past the coated media, let stand for 5 seconds, then expel completely (Don't worry about drying the bed, or introducing air. Flow is tangential to the surface and it will not affect binding as it would in a packed bed).
- 4. Load sample no further than the top of the coated surface. Use less if desired. Do not cycle the liquid. Pull liquid in, stop the flow, and let it stand for 5 seconds to allow diffusion of the sample to the wall. Expel excess sample liquid back into the sample container for subsequent assays (Note that the capacities of a SuproTip are low to promote full recovery of sample (ca. 25 ng peptide by C18 RPC). This means there is an upper limit to the concentration that can be obtained by trace enrichment!).
- 5. Rinse the SuproTip bound sample with an intermediate solvent weak enough to prevent movement of the desired component, but strong enough to remove unwanted salts, detergents or impurities. Use one <u>bed</u> volume as with the loading step. Wait for the impurities to move into the liquid, prior to expelling them. Repeat as necessary.
- 6. Elute sample in desired volume of mobile phase (100% water for HILIC, 70-95% MeCN or MeOH for RPC; H₂O with 5mM buffer for HIC; H₂O with 20% HOAc buffer for WCX; ; H₂O with 1% NH₄OH buffer for WAX, MeOH for silica normal phase, EDTA for IMAC.). If concentration is desired, use less volume in elution than in the loading step and pull this liquid up past the sample limit 2-3 times to rinse the surface. Elute a second time if desired.

OBSOLETE

SuproTipTM **IEX Instructions:**

Directions:

- 1. To begin wash with several Tip volumes of 50:50, MeCN: PrOH. Repeat 2-3 times. This will reduce the low MW ions in ESI-MS and will activate the media.
- 2. Wash with 100% water. Be sure to remove all of the activation solvent, as it will promote elution of sample during the binding step.
- 3. Condition SuproTip media with two pipette volumes of binding solvent (5mM HOAc for IEX.). Pull liquid into tip past the coated media, let stand for 5 seconds, then expel completely (don't worry about drying the bed, or introducing air. Flow is tangential to the surface and it will not affect binding as it would in a packed bed).
- 4. Load sample no further than the top of the coated surface. Use less if desired. Do not cycle the liquid. If sample contains significant amounts of salt, dilute it to less than 80mM salt and trace enrich the sample in the Tip to concentrate it by repeatedly loading it. Pull liquid in, stop the flow, and let it stand for 5 seconds to allow diffusion of the sample to the wall. Expel excess sample liquid back into the sample container for subsequent assays (Note that the capacities of a SuproTip are low to promote full recovery of sample (ca. 25 ng peptide by C18 RPC). This means there is an upper limit to the concentration that can be obtained by trace enrichment!).
- 5. Rinse the SuproTip bound sample with an intermediate solvent weak enough to prevent movement of the desired component, but strong enough to remove unwanted salts, detergents or impurities. Use one <u>bed</u> volume as with the loading step. Wait for the impurities to move into the liquid, prior to expelling them. Repeat as necessary.
- 6. Elute sample in desired volume of mobile phase solvent (20% HOAc for WCX or 1% NH₄OH for WAX.). If concentration is desired, use less volume in elution than in the loading step and pull this liquid up past the sample limit 2-3 times to rinse the surface. Elute a second time if desired.