

## Desalting and Ion Exchange Procedures

### 1 – Desalting column

- Cut ~15-20 cm of 150 x 360  $\mu$ m capillary (can use 250 x 360  $\mu$ m if getting very slow flow or have lots of peptide).
- Put a frit in one end and pack with 5 cm Poros R3 (can use C18 but clogs easier). Same procedure as standard column but use R3.
- Condition with 500 fmol angio on the off-line HPLC.
- Wash with 50  $\mu$ L 1% HoAc.
- The sample can be loaded but pH needs to be 2-4.
- Note: If slows too much during loading or washing then dry slightly and put a frit in the other end so the column can be reversed.
- Wash with 200  $\mu$ L 1% HoAc.
- Elute with 200  $\mu$ L 80% ACN/1% HoAc.
- Dry completely and reconstitute with 1% HoAc.

### 2 – SCX column

- Cut ~15-20 cm of 150 x 360  $\mu$ m capillary (can use 250 x 360  $\mu$ m if getting very slow flow or have lots of peptide).
- Put a frit in one end and pack with Whatman SCX in 1M ammonium acetate (5-6 cm). Rinse for 5 minutes with 1M ammonium acetate.
- Condition as follows:
- Rinse column with 50  $\mu$ L 1% HoAc.
- Rinse column with 50  $\mu$ L 1M ammonium acetate.
- Load 5  $\mu$ L of a 1:50 dilution of the stock BSA digest.
- Let sit on column overnight.
- Elute with 200  $\mu$ L 1 M ammonium acetate.
- Rinse column with 200  $\mu$ L 1% HoAc.

### 3 – SDS or non-ionic detergent removal

- Make and condition a desalting column.
- Check that pH = 2-4 in sample and load 50% (generally 5% acetic acid by volume – up to 10% but no more; if necessary this may be changed to 3-5% TFA for difficult samples).
- Rinse column with 200  $\mu$ L 1% HoAc.
- Elute with 200  $\mu$ L 80% ACN/1% HoAc.
- Dry sample completely and reconstitute in 20-50  $\mu$ L 1% HoAc.
- Note: Rinse the column with 200  $\mu$ L 1% HoAc. Can be used again on 1-3 similar samples from the same investigator most of the time but not if column slowed.
- Get a conditioned SCX column.
- Load all the sample from the desalting column (elution fraction).
- Rinse with 200  $\mu$ L 1% HoAc.
- Elute with 200  $\mu$ L 1M Ammonium acetate and dry completely.
- Reconstitute the sample in 20  $\mu$ L 1% HoAc.
- Note: Rinse the column with 200  $\mu$ L 1% HoAc. Can be used again on 1-3 similar samples from the same investigator most of the time but not if column slowed.
- The procedure can be done with ion exchange fractions instead of batch elution. If doing this, it is important that the ammonium acetate be pH = 4-4.5 (using acetic acid). The fractions (200  $\mu$ L) are 1, 2, 5, 10, 25, 50, 100, and 1000 mM ammonium acetate (also keep the wash after the first 10  $\mu$ L).