Tips for acid elution of peptides

(Modification of protocol described in Raghavan et al. and references therein).

1) Don't use pipetman yellow tips -- use white tips with filters that are used for sterile tissue culture or PCR (yellow tips have contaminant in them).

2) Use Centricons with a MW cutoff of 3000, not 10000 (the Centricon 3000's are made from a better polymer).

3) Centricons can fail. Cenck Amicon's limit for g-force on centricons and do spins a bit below maximum speed.

Protocol

1) Wash centricon first three times with 2 ml of 25 mM MES, Ph 6.0 (all buffers and water should be HPLC grade, e.g. from Baker).

2) Wash centricon twice with 2 ml washes of 10% acetic acid (highest grade).

3) Wash again with MES buffer at least once to neutralize.

4) Load protein sample in ~100 µl.

5) Add 1 ml 10% acetic acid. Invert centricon into prewarmed heating block (70˚C) for 15 minutes. Let it reflux.

6) Invert carefully (pipet material from cap back into centricon).

7) Spin to collect eluted peptides.

8) Now have ~2 ml peptide material in acid. Speed vac to reduce volume to 50 µl. Either analyse by pool sequencing or try to separate peptides by HPLC. Don't freeze eluted peptide material.