Post-FASP Strong Anion Exchange Separation (SAX)

Adapted from Wisniewski et al, J Proteome Research, 2009

Materials

1. **Stock solution of Britton & Robinson Universal Buffer (BRUB)** (Britton, H.T.K and Robinson, R.A, *J. Chem. Soc.,* 1931, 1456-1462)
    a. Prepare a 5x stock solution containing 0.1 M acetic acid, 0.1 M phosphoric acid and 0.1 M boric acid.
    b. Adjust with NaOH to the required pH. For standard FASP processed sample, the following pH values are recommended: pH 3, 4, 5, 6, 8 and 11.
    c. Before use, dilute 5-fold in DDW. Recommended amounts are ~120µl diluted buffer per sample from buffers pH-3,4,5,6, 8, and ~500µl diluted buffer per sample from buffer pH-11.
    d. Add NaCl to the 1x pH-3 solution to a final concentration of 0.25M.
2. **Buffer B:** 80% (v/v) ACN, 0.5% (v/v) acetic acid.
3. **Buffer A:** 0.5% (v/v) acetic acid.
4. **Anion-Exchange tip-column (in pipette tip) (SAX tip)**
    a. Stack 6 layers of Empore/Disk Anion Exchange (Varian, 1214-5012) in a 200µl pipette tip. Prepare 1 SAX tip for each sample.
    b. Cut the top off the SAX tip (3-4mm) so it can conveniently be centrifuged.
5. **StageTip**
    a. Stack 3 layers of Empore/C18 in a 200µl pipette tip (→ stageTip). Prepare 6 stageTips for each sample (1 for each pH), and label them properly.

Sample preparation

1. Elute peptides in FASP procedure or equivalent.
2. Verify the pH of each solution (after dilution) and correct with NaOH or BRUB.
3. Dissolve 20-50µg peptides with up to 200µl buffer 1x pH-11 and ensure pH is higher than 11 (correct with NaOH if necessary).

Anion Exchanger tip-column (SAX tip) activation

1. Assemble the tip-column in the eppendorf tube lid or adapter.

2. Wash with 100µl Methanol. Centrifuge at 3000 x g for 3 min. Ensure solution is not retained on top of the filter.
3. Wash with 100µl 1 M NaOH.
4. Wash **twice** with 100µl 1x pH-11 buffer.

StageTip conditioning

1. Assemble the tip-column in the eppendorf tube lid or adapter.
2. Wash StageTip with 100µl Methanol. Centrifuge at 1000 x g for 2 min. Ensure solution is not retained on top of the filter.
3. Wash StageTip with 100µl Buffer B
4. Wash StageTip twice with 100µl Buffer A

Sample loading and separation

1. Assemble the first (highest pH) StageTip-Sax tip apparatus as depicted in the drawing.
2. Load the diluted sample. If the sample volume exceeds the volume in the tip, you may perform the loading several times.
3. Centrifuge at 3000 x g for 3 min.
4. Add 100µl 1x pH-11 buffer to the SAX tip and centrifuge at 1000 x g for 5 min.
5. Transfer the SAX tip to the next StageTip.
6. Continue with stages 4-5, eluting subsequently with 1x pH 8,6,5,4 and 3 buffer, each into its corresponding StageTip.
7. After each centrifugation step, make sure that no liquid remains above the filter.
8. Wash the StageTips with 100µl Buffer A.