**SDC Solution Digestion Procedure**

from Humphries, et.al., *Nature Protocols*, 2018, (13), 1897-1916

**Reagents Needed**

1. 4% (w/v as g/100mL) Sodium Deoxycholate (SDC) in

100mM Ammonium Bicarbonate (Ambic), pH8 or 100mM Tris-HCL, pH 8.5

1. 5M Potassium Hydroxide (KOH)
2. Reduction/Alkylation Buffer

400mM Chloroacetamide (CAM)/100mM Bond Breaker TCEP, pH 7 in Water

Add KOH solution to adjust pH to ~8, if needed

1. Trypsin/LysC or Trypsin; sequencing grade
* Note – Solutions should be made fresh

**Day1**

1. Determine protein concentration by assay of choice. If concentration is sufficiently high (>10mg/mL) you may take an aliquot for digestion otherwise, proteins should be precipitated using Acetone, TCA or CL3CH:MeOH.
2. Re-suspend protein sample in 4% (w/v) SDC/100mM Ambic to 270uL. Solution may be cloudy and may show some undissolved protein but there should be no cellular debris. Centrifuge, if necessary, to pellet debris and move supernatant to a clean tube. Following digestion any cloudiness should disappear.
3. Add 30uL of Reduction/Alkylation buffer and heat at 45C for 5min with agitation to 1400rpm in the Eppendorf ThermoMixer.
4. Remove sample from ThermoMixer and allow to cool to room temperature.
5. Add Trypsin/Lys C enzyme mix or Trypsin at 1:100 ratio (enzyme to protein). Incubate in the Thermomixer at 37C with agitation to 1500rpm overnight.

**Day2**

1. Remove digests from heat and allow to cool to room temperature.
2. Extract SDC by mixing digests with an equal volume of 100% Ethyl Acetate and mix. Acidify to 0.5% TFA (pH~2) and mix. Centrifuge at 15,700 x g for 2min.
3. Upper layer should contain SDC and Ethyl Acetate, aqueous lower layer should contain peptides. Remove aqueous layer to a fresh tube and check pH, adjust to ~2 as needed using trifluoroacetic acid.
4. It may be necessary to dry samples briefly in a speedvac to remove residual ethyl acetate which will interfere with c18 clean up.
5. Desalt using c18 StageTips or SepPaks and dry. Proceed to MS analysis or freeze.