Protocol for in-solution Trypsin Digest (generic)

NOTES

- The following items of electrical equipment are used in this procedure. You should ensure that you have been instructed in their correct use prior to carrying out this procedure.
  - Heating Block
  - Thermomixer
  - Bench-top microfuge
  - Speedvac
- Individual samples can be adjusted to 50 µg in 50 µl 0.5M TEAB.
- Use Protein LoBind Tube, (0.5ml, Eppendorf)
- Digested and de-salted samples are re-suspended in an appropriate volume of Buffer A (0.1% Formic Acid) to give a final concentration of approximately 0.5 - 1 µg/µl for the Thermo Q Exactive.
- Re-suspended digests should be transferred to blue lidded MS vials and labeled.

REAGENTS

0.1% Formic Acid (Fluka, T4564-10ML-F)

- Prepare 1% from bottle (10 µl Formic Acid in 1 ml HPLC water)
- Dilute 1 in 10 for 0.1% (100 µl 1% + 900 µl HPLC water)

50 mM TCEP (Sigma, C4706-2G) *(Prepare fresh)*

- Mol wt. 286.65
- 0.14 g in 10 ml HPLC water

200 mM Iodoacetamide (Sigma, I6125-10G) *(Prepare fresh)*

- Mol wt. 184.96
- 0.37g in 10 ml HPLC water

Trypsin (Promega, V5111) *(Prepare fresh)*

- Each individual trypsin vial is re-suspended carefully in trypsin reconstitution buffer. 20 µg per vial so re-suspend each vial in 20 µl of buffer to give 1 µg/µl
- Individual vials are pooled into a single Eppendorf tube
- Trypsin is incubated at 30°c for 15 minutes before use (see trypsin application note)

METHOD

Day 1

- To 50 µg sample add 3 µl of 50mM-TCEP, incubate at 60°c for 1 hour
- Add 3 µl of 200 mM IAA and incubate at room temperature in the dark for 30 mins
- Add 5 µl of Trypsin (1 µg/µl) and incubate at 37°c overnight

Day 2

- Stop the digest by adding 1-2 µl TFA to each sample
- Spin digests at 15,000 rcf for 10 mins, collect supernatant and dry under vacuum in a Speedvac. (If multiples of the same sample are digested they can be pooled and re-aliquoted before drying down.)
- Re-suspend dried digest in Sample Prep. Solution (0.5% TFA) and proceed straight to ZipTip protocol for de-salting.
- For LC-MSMS analysis generally a minimum volume of 6 µl is required per injection onto the Q-Exactive at a concentration of 0.5 - 1 µg/µl. You should prepare sufficient for two injections, a minimum of 15-20 µl will allow for pipetting loss.