Sample Preparation Protocol - BioAccord 1 Intact Protein Analysis

Please aim for a protein concentration in the range **10** - **50** μ **M**. Depending on the ionisation of your protein, you may need to adjust the final concentration. Do not submit samples which have a concentration greater than **100** μ **M** as you will likely damage the instrument.

Over concentrated samples will be flagged to MS Staff and will lead to **increased chemical noise**, **poor mass resolution**, **blockage in the sample delivery lines and contamination of the mass spectrometer**. Please make sure that there are no hard particles in the solution or precipitation at the bottom of your sample vial, and that the solution is not jelly-like or cloudy.

Only **standard 2 ml mass spec sample vials** with a **pre-slit septum** on the top of the lid should be used. The vials are available from CRL stores. No taller vials or vials with hard lids can be used.

BioAccord 1 uses **electrospray ionisation** which is only **compatible with volatile organic solvents and water.** Samples have to be cleaned of salts: **high salt concentrations are not compatible with ESI.** Please follow the protocol below for sample preparation:

- 1. Purify your protein using a suitable filter (eg. Amicon MWCO or Bio-Spin column) to remove salts and impurities. LC purification would also be appropriate.
- Resuspend your protein in acidified aqueous acetonitrile (ideally 5% MeCN_(aq) + 0.1% FA) to a final protein concentration between 10-50 μM.
- 3. Place the solution in **a standard 2 mL mass spec sample** vial with a screw cap lid and pre-slit septum on the top (available from stores). Other 9mm insert vials such as Thermo Scientific SureSTART microvials may also be used so long as they have pre-slit screw cap lids and are the same height as the standard 2 mL vials.
- 4. **Do not use Tetrabutyl ammonium (TBA) or Trifluoroacetic acid (TFA) in your samples** (or any other ion-pairing agents) these will contaminate all subsequent samples run on the system.