Sample preparation for LC-MS/MS - Guidelines

Good sample preparation is fundamental to successful mass spectrometry and there is no one-size-fits-all method. Proteins must first be extracted from the sample in question and solubilized, before they can subsequently be digested to peptides for mass spectrometric analysis. However some buffer components (in extraction and lysis buffers) can cause problems for down-stream mass spectrometry. Where contaminants such as inorganic salts and detergents are present in a sample they must be removed before submitting to the Mass Spec Facility.

Contaminants Incompatible with Mass Spectrometry

A. Detergents, Polyethyleneglycol (PEG) and other Polymers

Detergents are frequently used during sample preparation in extraction and lysis buffers, for example SDS, NP-40, TWEEN, and Triton X-100. Polyethylene glycol (PEG) is present in many of these detergents, but is also present in dish-washing liquid. The presence of PEG in a sample causes ion signal suppression on the mass spectrometer. Additionally detergents can reduce the efficiency of trypsin in the subsequent peptide digestion step, and they can be extremely difficult to remove from both the HPLC column and mass spectrometer.

- Reduce the risk of contamination by ***not*** using detergent to wash glassware
- Use HPLC grade solvents (including water)
- Use good quality branded plastic consumables (eg. Eppendorf tubes)
- Do not pipette directly from stock acid bottles. Decant first to smaller containers.
- If possible design your sample preparation to avoid using detergents or use mass compatible ones instead such as RapiGest.

B. Cell debris, insoluble material or particulates

These can become trapped in the chromatography system, building up over time and causing pressure irregularities and distortions in the chromatography.

C. Cryoprotectants: DMSO, Glycerol, DMF etc

These are viscous and can be detrimental to the chromatography system, They can interact with tubing and other machine parts.

Volatile buffers

Where possible you should avoid inorganic salts and non-volatile buffers in the final sample preparation steps e.g. Tris, phosphate. Non-volatile salts and buffers can form adducts, suppress ionization of your peptides, and affect the chromatography. Volatile buffers such as ammonium bicarbonate or TEAB will leave no residue when the sample is dried down prior to final re-suspension for loading onto the mass spectrometer and should be used in preference.

Clean up methods

SDS-PAGE

SDS-PAGE can be used both to fractionate and to cleanup a sample for mass spectrometry.

- Use pre-cast SDS-PAGE gels, ready made buffers and commercial stains where possible
- Use freshly made buffers (communal buffers are a source of keratins)
- Keep staining trays covered (don't use trays that have been used for Western blots)
- Wear nitrile gloves (not latex) and a lab coat while handling the gel

Protein Precipitation

A number of solvents can be used for protein precipitation and protocols for each can be found on-line. After precipitation try to avoid drying the pellet completely as it may be difficult to re-suspend subsequently. Ideally use a volatile buffer such as ammonium bicarbonate or TEAB (Triethyl ammonium bicarbonate). Examples of precipitants are -

- Acetone (in 4 x volume of 80%)
- Chloroform-Methanol (in x volumes of 4:1:3::MeOH Chloroform:H_2O)
- TCA (to a final concentration of 20%)

Buffer Exchange

Buffer exchange is particularly suited to the removal of detergents. The FASP protocol (Filter Aided Sample Preparation) combines buffer exchange with the subsequent trypsin digestion and is recommended for the removal of SDS from samples. All steps are performed in Vivacon filter units.

De-salting with ZipTips®

All tryptic digests should be processed with C_{18} Zip-tips before drying down and re-suspending in Buffer A (0.1% Formic Acid) for LC-MS/MS analysis. Tips can bind up to a maximum of 5ug of protein/peptide sample.