**Protein Sample Preparation Basics for LC/MS Analysis**

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**Before you start your project!!!**

* All users must contact Dr. Kakhniashvili and discuss specific project details before submitting samples to the PMC

**The quality of prepared samples is the top priority**

**The quality** of prepared samples may be affected by:

1. External contaminants
2. Reagents used for sample preparation/processing
3. Abundant sample proteins

**External Contaminants**

1. Contaminants may be introduced at several steps during sample preparation. Some contaminants may ruin MS analysis. Investigators who do not follow these recommendations for sample preparation will be charged for MS analysis, even their requested experiment fails during LC/MS analysis.
2. **Keratins** are the most common contaminating proteins. The presence of keratins or other contaminant proteins is a problem limiting identification of target proteins. Keratin sources include **air, dust**, skin, hair, clothes, some chemicals – **keratins are everywhere, and on everything**. Keep bench/working surface clean and samples covered or protected as much as possible.
3. **Serum proteins** (BSA, immunoglobulins, etc.) are frequently observed contaminant proteins often originating from cell culture media (when cell pellets are not thoroughly washed with PBS). Thoroughly wash cell pellets with PBS before proteins are extracted.
4. **Glycerol, PEG and similar polymers** severely affect LC/MS analysis, even if present at very low concentrations. In addition, polymers tend to stick to an HPLC column and may ruin it. Sources of these contaminants include soaps/detergents, hand-creams, some reagents and plastics.
5. **Wear powder-free gloves** at all times during sample preparation. **Do not** touch contamination sources (see above) with outer working surface of gloves; if you come into contact, change gloves.
6. **Use new** disposable **tubes, pipettes, tips.** **Thoroughly wash/clean anything** un-disposable that will come into contact with your sample (i.e. centrifugal tubes, gel apparatus, staining trays, gel excising implements, gel imaging or storage equipment, etc.). Cleaning is required to remove keratins and other contaminants.
7. **Do not** use plastic or glassware previously exposed to washing detergents. Detergents interfere with LC/MS analysis.
8. **Use new** molecular mass cut off filter devices; **wash** to remove filter preservatives before use.
9. **Tubes/vials/Parafilm** may be a source of extractable contaminants detrimental to LC/MS analysis. Do notuse colored tubes or coated (‘low-binding’) vials/tubes or vials with rubber O-ring or gasket. Use only 1.5ml transparent plastic tubes resistant to acetone (e.g. Eppendorf brand, polypropylene).
10. **Plastic pipettes/tips/tubes** – DO NOT use plastics to handle concentrated acid stocks used for MS experiments. Plastic extracts accumulate, contaminate the stocks and, then downstream solvents, solutions, and samples.
11. **If you are unsure of your ability to prepare a sample according to these guidelines, please contact the Core Director about options for the Core to prepare your samples*, or collaborate with another laboratory who are experts in proteomics sample preparation to assist with your sample preparation.***

**Reagents Used for Sample Preparation/Processing**

Preparation/processing of protein extracts for LC/MS analysis may involve buffers, salts, enzymes, inhibitors, detergents, denaturing/chaotropic agents, reducing/alkylating/peptide reactive agents, and, sometimes, DMSO (dimethyl-sulfoxide), DMF (dimethyl-formamide), or stabilizers such as glycerol, or PEG polymers. These components (except for enzymes) are usually present at concentrations at least an order of magnitude higher than the analyzed protein/peptides and most of them are extremely detrimental to LC/MS analysis even at low concentrations; some of these agents may interfere with specific step(s) of sample processing as well. Therefore, they **must be removed** before LC/MS analysis at appropriate processing steps.

**Necessary processing components,** including antibodies (for IP) and proteolytic or other processing enzymes, should be used **at sufficient, but minimal, concentrations.**

**Any undissolved,** **particular matter** will clog, and potentially irreversibly damage the HPLC column and, therefore, **must be removed** before LC/MS analysis (e.g. by centrifugation).

**Detergents** (both ionic and non-ionic) severely interfere with both LC and MS parts of analysis; some of them may, as denaturing agents, interfere with the proteolytic digestion step as well. Detergents are usually difficult to remove from digested protein samples and should be used in the early steps of processing, or even avoided, where possible. Detergents can be successfully removed before proteolytic digestion of proteins using FASP columns or through protein precipitation (acetone precipitation, for example).

**Salts/Buffers** decrease sensitivity, greatly complicate MS analysis, and damage essential elements of MS instrument including its ion optics. Salts, buffers, other small hydrophilic components can be removed through a simple desalting process using ZipTips or equivalent before LC/MS analysis. **ZipTip columns are available for resale in the PMC.**

**Stabilizers,** e.g. glycerol, or PEG polymers, severely interfere with LC/MS analysis even at very low concentrations and are difficult to remove from prepared samples. In addition, PEG polymers tend to stick to an HPLC column and may ruin it. Usually, use of protein stabilizers is not necessary for sample processing involving proteolytic digestion and **should be avoided**.

**DMSO, DMF** interfere with MS analysis. Usually, they are not necessary for sample processing involving proteolytic digestion and **should be avoided**.

**Protein Extracts Containing Extremely Abundant Proteins**

Analysis of medium and low abundant proteins is extremely difficult/impossible in the presence of highly abundant proteins (e.g. hemoglobin in red blood cells, albumin in blood plasma); selective depletion of abundant proteins (to at least average abundance level) BEFORE proteolytic digestion of protein extracts facilitates analysis of the other proteins.

**PMC STANDARDS: Quality Control (QC) Check**

It is now possible to run a very small amount of your purified, undigested sample on an Agilent protein chip, which are available in the MRC. This Agilent run will not only determine the protein concentration of your samples, but will also establish if your samples are degraded or contain a particular protein at high abundance that needs to be removed. **All samples to be processed in the PMC MUST be run on the Agilent Bioanalyzer in the MRC before samples will be subjected to LC/MS analysis.**