Common Contaminants in Proteomics Mass Spectrometry Experiments

Various laboratory chemicals commonly used in biochemical and molecular biology laboratories can drastically interfere with proteomics experiments and reduce the number of proteins identified in your sample. YOU SHOULD AVOID THESE CHEMICALS WHEREVER POSSIBLE and talk to the staff of the Central Proteomics Facility about alternatives or how to minimse/remove them from your sample if you are using them.

1) **Polyethyleneglycol (PEG) and detergents containing PEG (common pegylated detergents include; Triton X-100, Tween, NP-40…). (MOST IMPORTANT TO AVOID!! – BE AWARE THAT TRACE PEG CONTAMINATION OFF CONTAMINATED SURFACES CAN BE A PROBLEM IN PROTEOMICS EXPERIMENTS). PEG contamination shows up in the mass spectrum as peaks separated by 44 Da.**

   THIS IS ONE OF THE MOST COMMON INTERFERING MOLECULES WHICH CONTAMINATE PROTEOMICS SAMPLES as the detergents are widespread in all biomolecular research labs. TRY TO AVOID USING THESE DETERGENTS AND IF YOU MUST USE THEM TALK TO THE CPF BEFORE SUBMITTING THE SAMPLES. If they are present in your sample they will dramatically reduce the quality of your data. NOTE: running the samples on a simple SDS PAGE gel will remove most PEG/PEG containing detergents from a sample.

   Even if you are not using these detergents yourself be VERY AWARE that lots of things in your lab are probably contaminated with PEG containing detergents eg,

   - **Glassware previously used for buffers might be contaminated – SOLUTION always use new glassware and ONLY use it for proteomics experiments.**
   - **Glassware sent for washup or washed in a dishwasher (washing up liquid contains PEG) – SOLUTION always clean your own glassware using only very hot water and/or organic solvents and rinse glassware at least 10 times with milli Q grade water.**
   - **Use the highest grade reagents possible – use HPLC grade solvents and analytical grade reagents. We use Fluka analytical grade reagents. DO NOT USE COMMUNAL LAB CHEMICALS - YOU DO NOT KNOW WHAT SOMEONE ELSE HAS CONTAMINATED THEM WITH.**
   - **DO NOT STORE ORGANIC SOLVENTS IN PLASTIC TUBES – contaminants leach out of the plastic with storage. SOLUTION use new glass bottles or disposable glass scintillation vials**
   - **USE EPPENDORF brand microcentrifuge tubes – We use Eppendorf branded tubes without problems. We don't recommend any other tubes.**
   - **Have your pipettes ever been used for pipetting detergent containing solutions? Ideally you should clean them or even better have a set which is only used for proteomics.**

   IF YOU DO NOT KNOW WHAT, WHO or WHERE A PIECE OF GLASSWARE, PIPPETTE or LAB CHEMICAL HAS BEEN PREVIOUSLY USED YOU SHOULD NOT BE USING IT IN A PROTEOMICS EXPERIMENT WITHOUT CLEANING IT VERY THOROUGHLY. IT IS BETTER TO BUY NEW

REAGENTS AND LAB GLASSWARE AND USE THEM EXCLUSIVELY FOR PROTEOMICS EXPERIMENTS.

2) **Keratin is a common protein contaminant in a lot of proteomics samples. Keratin from your skin and hair is present in dust in the lab or can fall off you into your sample. It is important to try and minimise keratin contamination by working carefully and cleanly.**

   - **Be aware that any surfaces, glassware or chemicals exposed to the lab atmosphere for more than a few minutes will be contaminated with keratin as dust from the atmosphere settles. SOLUTION – wash everything before use and then keep everything covered with tin foil. Always close the caps on your eppendorf tubes. Everything should be stored between uses in cupboards in sealed plastic bags or covered with tin foil.**

   - **SDS PAGE – Use precast gels such as NuPage gels from Invitrogen and use their readymade buffers, loading dye etc to minimise keratin contamination. Gel tanks are a common source of contaminants. IF YOUR GEL TANK HAS BEEN LEFT TO DRY BY THE SIDE OF THE SINK FOR SEVERAL DAYS IT IS NOW CONTAMINATED WITH KERATIN FROM DUST**

   - **Always wear gloves when working with material for proteomics and work quickly. If you can work in a laminar flow hood it will help minimise dust and keratin but does not negate the need to be careful.**

   - **When staining your gel do you leave the staining tray uncovered on the shaker? – cover the staining tray with tin foil at all times and wash the staining dish well before use**

   - **Communal lab chemicals, eg loading buffers, lab buffers etc are common sources of keratin and dust.**

3) **Polysiloxanes ($[R_2SiO]n$) are a rare contaminant in proteomics samples and typically show up as peaks separated by 76 Da in the mass spectrum. Their source is typically from siliconized surfaces and plasticware where the coating is used to minimise adsorption of liquid to surfaces eg in high-recovery tips. We don't recommend using siliconized products. Buy high-quality, virgin plastic tips from a company like Eppendorf.**