

Minimizing Contamination in Mass Spectrometry and Proteomic Experiments

Many common laboratory chemicals used in biochemical and molecular biology research can significantly interfere with mass spectrometry experiments causing signal loss and can contaminate the instruments. The biggest culprits are high salt concentrations, PEG, and keratin.

High Salt

High salt concentration suppresses ionization and should be avoided or removed prior to mass spec analysis. Any salt with Na or K (PBS, K_2HPO_4 etc) will suppress ionization. Only volatile salts (NH_4 salts like ammonium acetate, ammonium bicarbonate) are suitable for mass spectrometry and typically less than 100 mM concentration. The tolerance for salt depends on the ionization method used (MALDI is more tolerant to salts and buffers), the size of your molecule (the larger your molecule the more intolerant to salts and buffers it becomes) and the concentration of your analyte. Low concentration of analyte is very intolerant to even low salt concentrations. We have ways of desalting your sample if your sample contains salts and buffers, just be sure to indicate your sample conditions on the form so appropriate steps can be taken.

Polyethyleneglycol (PEG)

PEG is everywhere! It is strongly ionized by ESI or MALDI and can swamp out signal from the analyte of interest. PEG has a common repeat of 44 Da in mass spectrum. Many detergents contain polyethoxylated surfactants/detergents including dish-washing soaps. Triton X-100, Tween, NP-40. These are pegylated detergents and should be avoided. PEG is also used to coat Chem-wipes so if you are wiping down your lab bench/glassware etc with Chem wipes you are smearing PEG everywhere. Removal of PEG for small molecules is very difficult and the best approach is to just avoid it (see tips below). If these detergents are used for protein samples the best way to remove them is by SDS-PAGE. Trace amounts of PEG can ruin a mass spec experiment. And when I say trace I mean trace and even less than what you think trace.

- Glassware previously used to make buffers can be contaminated with PEG. Keep glassware used for buffers separate from non-buffer making glassware.
- Dish wash soap contains PEG. Either do not use or rinse glassware with very hot water followed by an organic solvent rinse (like IPA).
- Use only HPLC grade solvents and Fluka analytical grade reagents.
- Do not store organic solvents in plastic tubes. PEG contaminants can leach out of plastic tubes. Use new glass bottles or disposable glass scintillation vials (with Teflon lids)
- We recommend Eppendorf brand microcentrifuge tubes.
- Use disposable pipettes. Pipettes that have been used to pipette buffers are contaminated.

- Avoid siliconized surfaces or plastic ware. We observe polysiloxanes (repeat of 74 Da).

Keratin

Keratin is a common protein contaminate. Keratin originates from skin and hair but is present in dust in the lab.

- Any surface in the lab including glassware, reagents, chemicals exposed to the lab atmosphere for more than a few minutes will be contaminated with enough keratin to be detected by mass spec sequencing. Wash everything with organic solvent (IPA) before use and keep everything covered with foil, or in sealed plastic bags or bottle caps. Anything left to dry at the side of the sink is contaminated with keratin.
- SDS-PAGE – We strongly recommend that you use pre-cast gels (NuPAGE from Invitrogen) and use ready-made buffers, loading dye etc. However, if using premade buffers, take care not to contaminate your buffer stock with contaminated pipettes and glasswear.
- Gel tanks are a common source of keratin contamination.
- Always wear gloves and change them frequently. If you answer the phone or pick up a pen with your gloves on, your gloves are now contaminated.
- Working in a laminar flow hood will help minimize dust and keratin but not eliminate it if your glassware is dusty.
- When staining your gel cover the tray with foil or wrap at all times. Be sure to wash the staining dish well (rinse with IPA)
- Communal lab chemicals (loading buffer, lab buffers etc) are the most common sources of keratin. Do not use – make fresh every time.