Creating and Maintaining a Keratin-free Environment

Keratin FAQs

What are keratins?

Keratins are a class of structural proteins found in all vertebrates. There are over 30 types of keratin proteins.

What are common sources of keratin contamination?
Keratin may come from your hands, your hair, your angora or wool sweater, and from the air. In fact, most of the dust particles in our laboratory (and home) are from keratin.
What will keratin do to my proteomics sample?
When introduced into your sample, keratin will be digested and analyzed along with your protein. If your protein is present in small abundances compared to the keratin, the keratin peptides will overwhelm the analysis. In many cases, several keratin peptides will be detected by the mass spec, while only a single peptide of interest is detected.
When is keratin an issue?
If your protein is present in high quantities (darkly stained by coomassie), then keratin may be present in your mass spec data, but is unlikely to interfere with the identification of your protein. However, coverage may be less then if keratin were not present. If your protein is present in medium quantities keratin may or may not interfere, but will certainly be detected. Coverage will be less then if keratin were not present. If your protein is present in small quantities (ie visible only with Silver or Sypro), then keratin may prevent identification of your protein. Coverage will definitely be less then if keratin were not present.
How can I avoid contaminating my sample?
Follow the suggestions in this guide and be diligent about maintaining a keratin-free environment. Awareness of your surroundings and movement and knowledge of the consequences is the best defense you have.
What if I am not able to do all of the things you suggest? Can I still do a successful proteomics experiment?
Yes! Even if your protein is present in very small quantities, you can still have a successful experiment. Try to follow the suggestions as much as possible and use common sense.
Laboratory equipment and supplies
If possible, you should have laboratory equipment and supplies that are dedicated to proteomics. These include:
1) Laminar Flow Hood if possible. A plastic shield is also acceptable.
2) Pipettemen (1 ml, 200 ul, 20 ul, and either 1-10 or 0.1 - 2 ul)
3) Tips and plasticware that is dedicated. Filter tips are preferable. We label tip bags as "keratin-free" and store them separately.
4) Centrifuge (we use a shared centrifuge when necessary but have a mini-benchtop centrifuge in our hood as well)
5) Microfuge tubes
    a. Low retention tubes are recommended
    b. If you are unsure of your tubes, rinse them in HPLC grade Acetonitrile or Methanol, followed by a rinse in HPLC grade water
6) Incubator set at 37 degrees.
7) Vortex with attachment to hold microcentrifuge tubes.
8) Glassware that is dedicated to proteomics - no detergents if possible
9) Speedvac and/or lyophilizer. Make certain you know what other reagents are used in shared equipment. If possible, these should be dedicated. If not possible, you may want to wipe down the speedvac and centrifuge prior to adding samples.

Your working area
Since dust is a major source of keratin, make certain you are working in an area that is free from drafts and/or significant air flow. Although laminar flow hoods are ideal, you can also work behind a shield. If possible, designate a small area on the bench as "Proteomics Only". Supplies can be kept in this area as well. If you do not have a dedicated hood or working space, at the very least make certain that you take

some basic precautions: 1) Always be cautious when handling tubes. Always point the tubes away from your face and make certain your skin does not touch the tubes. 2) Wipe down your pipettemen with methanol before use 3) Use tips and tubes from unopened boxes/bags

Solvents and reagents

You must ensure that your solvents and reagents are of the highest grade possible. Remember that mass spectrometers are exquisitely sensitive instruments and can detect very small quantities of contaminants. In addition, small particulates can cause blockage of the HPLC. Some items that you will need to have on hand:

Urea
Ammonium Bicarbonate (ABC)
Dithiothreitol (DTT)
Iodoacetamide (IAA)
TCEP
4-vinyl pyridine
Trypsin, or other enzymes
HPLC grade water
acetonitrile
methanol
formic acid
trifluroacetic acid

Proteolytic enzymes

Your choice of enzyme depends on your protein sequence. In general, trypsin is used as a starting point, especially for unknown proteins. However, if you know the sequence of your protein, it is a good idea to perform an in silica digest using a software program such as PAWS or MSdigest at Protein Prospector . These programs will generate cleavage information to let you know if you are using the correct enzyme. Your cleavage product should generally be between 350 - 2500 Da. If it is larger, you may need to adjust your mass spectrometer settings. If it is smaller, you likely need to use another enzyme. Proteolytic cleavage patterns can have a profound effect on MS data and database search results. For more detailed information on protein digests including the effects of using specific proteolytic enzymes see Protein Digests

Gels

We recommend the use of pre-cast gels. If casting your own gels, make certain your reagents are of highest quality. Wipe glass plates with ethanol and cover if not used immediately. When staining, use nanopure (milliQ) water and staining boxes that are thoroughly cleaned and rinsed. We recommend the use of dedicated staining boxes and generally find a methanol wash followed by nanopure water to be sufficient. Rinse gloves prior to use as some contain residue that can be left on the gel. Try not to touch gels and, if necessary, touch only areas of the gels that do not contain protein. When imaging, place plastic wrap on the imaging screen. We usually place wrap on top of the gel as well. When excising protein bands or spots, use clean (new if possible) razor blades. Commercial spot cutters (robotic and manual) are also available. To avoid cross contamination, either use a clean blade for each protein, or rinse blade with methanol and wipe with a paper towel. If possible, perform behind a shield.