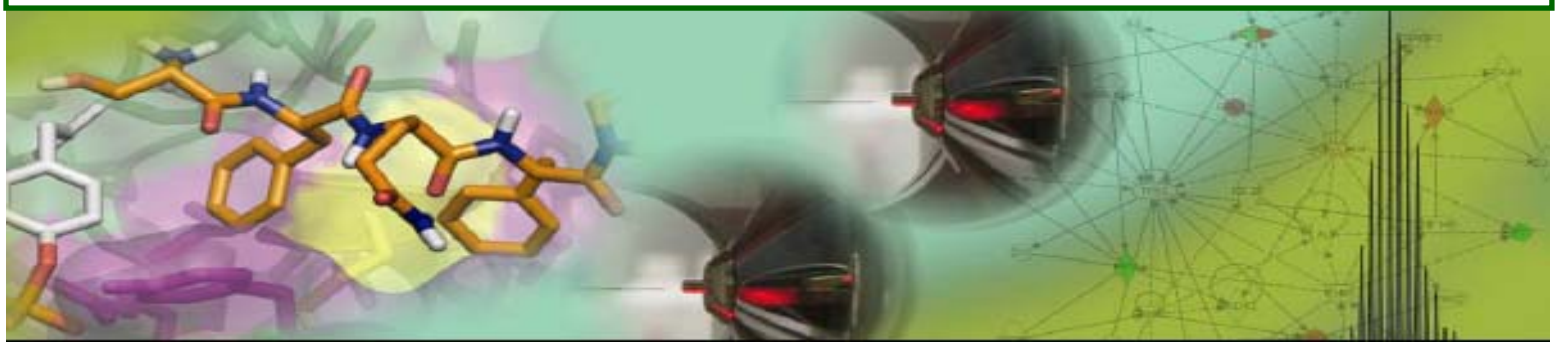
MS & Proteomics Resource

Yale School of Medicine
Keck Biotechnology Resource Laboratory



Application Note 1: Reduction of keratin levels for in-gel digestion and LC-MS/MS analysis

Keratin is one of the most abundant fibrous structural proteins that exist in human hair, skin, and nails (as well as in wool from mammalian animals and in silk from insects and spiders, etc.). Since ordinary dust constitutes most of these keratinous dead cells, keratin contamination poses as one of the most prolific problems that are often encountered in a laboratory setting performing MS based identification of proteins from biological samples.

To minimize Keratin contamination of the submitted samples, especially from polyacrylamide gels, and maximize success for experiments, the below guidelines should be followed:

- Please wear gloves and clean lab coats if necessary to minimize contamination from skin, nails, and natural fabrics like wool.

- Try to do the slicing/excising of the gels on a clean surface and in an area where there is little foot traffic to avoid dust/skin/hair getting on the gel apparatus and samples. Similarly, all of the gel apparatus, reagent bottles, vials, etc. should be kept clean and in an environment where minimal exposure to ambient atmosphere and dust exists. Consider staining and de-staining the gel in a brand new Petri dish that has been rinsed with de-stain.

- The polyacrylamide gel bands of interest should be sliced or excised with a “sterile”, clean sharp razor blade and should be handled with tweezers that have been rinsed with “fresh” methanol prior to use. It’s important that these rinsing reagents are dispensed from a “clean” source.

- After the gel bands of interest have been sliced or excised to a desirable width (i.e. 1-5 mm width), please place them inside of a 1.5 ml polypropylene eppendorf tube without any buffers or solvents. It is advisable to pre-rinse/vortex blank tubes with clean methanol prior to placement of the gel bands.