Sample Preparation for *In Situ* Enzymatic Digestion in SDS Page Gels

- Wear gloves, lab coat and work as dust free as possible.
- We recommend that gels be stained with Coomassie Blue (either R250 or G250). In the case of G250 (colloidal) Coomassie Blue, the sensitivity of staining appears to be about 25 ng (*i.e.*, 0.3 pmol) transferrin in a 0.5 mm thick gel. Currently, we do *not* recommend the use of silver staining. If you need to use silver stain contact me for a protocol.
- Scan the gel and send a copy of the image with the excised band circled and molecular weight markers labeled.
- Rinse all tubes with methanol and wipe down all surfaces with methanol. This is important to avoid keratin contamination.
- Wipe razor blades with methanol-soaked lint-free cloth
- Excise the band from the gel in such a manner as to avoid removing excess gel that does not contain any protein. 1mm cubes.
- Place the excised band in a clear Eppendorf tube and freeze. Do not dry it or leave the gel band in destain buffer or in any other liquid.
- Excise a similar size piece of "blank" gel (that does not contain any protein) and put it in a separate Eppendorf tube so that this blank section of gel can be used as a control to identify HPLC artifact peaks.
- Be sure to include as much information about the sample as you can such as species, type of preparation (nuclear extract, BALF) etc.

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