Guide to Silver Staining for Mass Spectroscopy

Note: Submission of Silver stained samples for protein identification REQUIRES pre-approval for the Protein Core Staff.

Use the protocol for Standard Coomassi stained gel pieces incorporating the following modifications for improved sensitivity:

In order to increase signal, reduce chemical noise and to minimize contaminants that tend to adversely affect our nLC columns, it is required for silver stained gels that you use pre-made gels. Concentrate your protein sample and load as much as you can into narrow deep wells of a 1.5 mm gels (max vol. Invitrogen Nupage 37ul, Biorad Ready Gel 50ul, Criterion 45ul). Reduce & Alkylate your samples prior to electrophoresis using the protocol "Reduction & Alkylation Protocol #1" (Sample_rednalk_Protocol#1). Invitrogen Nupage gels are preferred as they provide the cleanest digests in our hands, however the Bio-rad pre-mades are also acceptable. Use the narrow deep well for larger volumes. And pre-run the gel before loading your samples for about 10 min at 200mA. This will move any free acrylamide ahead of your sample preventing any acrylamide adducts.

Use only Invitrogen's Silver Quest (LC6070) staining kit as per instructions (Peirce Silver Stain for MS may also be used) and **keep development times to a minimum**. It is common for people to push development time thinking it is better if the band is darker, but in reality it reduces the amount of protein available for MS. The reason for this is that the protein that has been stained has bound silver which interferes with enzyme digestion, peptide extraction and ionization in the mass spec. What you are looking for is the 'sandwich effect' whereby the silver just stains the surface such that when you cut the gel slice there is a layer of stain on the top and bottom with a clear layer in between. This clear layer is where the usable protein will be found.

Silver stained samples(10-500fmole) must be submitted for nano reversed-phase LC MSMS (nRPLC MSMS).