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### **Sample Preparation and Submission of Coomassie Blue stained gel piece for MS Protein Identification and Analysis (11/08)**

The requirements for sample submission are stringent. We have designed and tested this process to insure a successful outcome. Please be aware that all of these requirements are directed at giving your sample the best chance of being correctly identified, with any remaining sample suitable for further analysis if necessary or desired. In general, the preparation of sample by the investigator is fairly straightforward. However, this process will not tolerate deviation from the prescribed method. Please remember that we have limited personnel in our proteomics section at the present time. Users must perform a large part of the sample preparation in their own laboratories or, if they lack access to specific pieces of equipment, in the core facility with our guidance.

It is recommended that you use pre-made gels such as Invitrogen Nupage, or Biorad Ready Gels, & that you Reduce & Alkylate your samples prior to electrophoresis using the protocol "Reduction & Alkylation Protocol #1" (Sample\_rednalk\_Protocol#1, available on our website "Download" page). Other types of 1D and 2D gels, ranging from 7.5% to 12.5%, can be used if discussed with our staff prior to running your gel. NOTE: PRE-RUNNING(10 min. 200mA) the gel with no sample to remove UNPOLYMERIZED ACRYLAMIDE before you load your samples is recommended. In general, higher percentage gels are recommended for proteins less than 30 kD (12% ) as this minimizes the loss of sample during staining, destaining, washing and subsequent in-gel tryptic digestion (if higher than 12% is required please discuss this with Proteomics Core Staff before proceeding). Samples need to be excised from the gel in the smallest volume possible. Gloves, sterile cutting blade & cutting surface are essential. A Laminar flow environment will help decrease keratin contamination. Ideally 1.0mm to 1.5mm thick gels should be used. Try to keep the protein in a single gel lane such that the band measures no more than 1mm x 1.5 mm x 4 mm. If the band is larger it will be treated, and charged, as two samples in the digestion phase of the experiment. If there are technical problems that prevent loading the sample in a single gel lane, we suggest you email or call the Proteomics Core with a short description of the problem as we may be able to provide assistance.

Quantitation is important and a requirement for sample submission. **A copy of the photograph** of your gel must be submitted before processing begins. From this photo we may be able to give you some idea of your chances of successfully identifying your protein or make recommendations that will lead to a successful analysis. To accurately estimate the # of pmoles in your protein sample you should run it with known quantities of two concentrations of unstained standards in adjacent lanes of the **SAME** gel (only one concentration is necessary for Coomassie stained gel bands). We recommend Invitrogen's Novex Sharp(LC5801) or Biorad's Precision Plus unstained molecular weight markers(cat#1610363). Densitometry can be used to compare the staining intensity of your sample to the intensity of the known quantities of protein standards. There is a laser densitometer and appropriate software in the core facility available 24/7. Since this estimate will determine procedures to be used for the entire experiment it is important that the estimate of the amount of protein submitted be as accurate as reasonably possible. Any divergence from this protocol or expectations beyond protein identification must be discussed in advance of sample preparation.

For staining, we recommend Invitrogen's Colloidal Coomassie Blue kit(LC6025) or Pierce GelCode Blue; the sensitivity of staining is about 50ng. Samples should be submitted in 1.5mL Eppendorf tubes clearly labeled. As a negative control for the process please submit a "blank" (a part of the gel from a lane that no protein was applied) portion of your gel about the same physical size as your unknown sample in a separate tube (there's no charge for the neg. control blank sample). If you have multiple samples from the same gel only one negative control is necessary. Experimental negative controls for which you expect data analysis are charged as regular samples. Please fill out the sample submission form as clearly and completely as possible. Every bit of information requested is important for the identification of your protein. Submission of samples for protein ID in any other form than Coomassie stained gel bands must have prior approval.

**This information is a guide, please speak to Steve Bobin or Susan M. Kennedy before proceeding to prepare samples for MS.**