



## **HARVARD** **MASS SPECTROMETRY & PROTEOMICS**

52 Oxford Street B247  
Cambridge MA 02138-2020  
617-495-4043

- **Please do not prepare, run gels or send samples without first discussing the project and prep procedures a scientist.** Optimum protocols can change frequently---one of the most significant determinants of the success of your analysis will be the preparation.
- We believe strongly that a fully reviewed project and preparation procedure ultimately leads to the most expedient and sensitive analyses.
- Enclosed is a form for each of the services offered at the Harvard Mass Spec & Proteomics Resource.
- Each sample must be accompanied by a completed form.
- The information you provide on these forms helps to ensure proper handling of your sample.
- You will need to send a hard copy of your PO via fax (617-495-1374), email ([hmf@harvard.edu](mailto:hmf@harvard.edu)) or with your forms and sample.
- For protein identification the HPLC MS/MS PROTEOMICS FORM should be completed.
- For intact MW determinations use the MASS SPEC FORM.
- For intact N-terminal sequence determination by Edman use the PROTEIN SEQUENCE FORM.
- Important information to include on your forms:
  - Sample Name
  - Your phone number and fax number.
  - Your billing information: PO number, billing address, and purchasing agent phone number.
  - The MW, quantitation (even if only an estimate), and method of quantitation.
  - The organism(s)/species of your sample.
  - For direct sequencing (*not* digests), be sure to specify the number of sequencing cycles desired.
- It is best to send samples via FedEx. If you choose to drop off samples in person please be advised that **drop-off hours are Monday through Friday, 10:00 AM to 3:00 PM.**

**INCOMPLETE / INCORRECT FORMS WILL DELAY YOUR SAMPLE**

# HARVARD UNIVERSITY

Mass Spectrometry and Proteomics Resource Laboratory

Current Date

## HPLC MS/MS and PROTEOMICS *Sample Submission Form*

Your Name:  Phone #:

Sample Name:  Fax #:

Email:

### *Billing Information:*

**Principal Investigator:**

Institution:

Billing Address:

Purchasing Agent Phone #:

Harvard/Affiliate's 33-digit Ac:count #:

Non-Harvard User's P.O. #:

### *Sample Information:*

MW:  Organism/Tissue Source:  Medium:  Gel Stain:

Estimated Weight (ug):  Estimated Amount (pmole):  Estimate Method:

### **Project Goal:**

- Identification via LC MS/MS
- Modification via LC MS/MS
- Quantitation via LC MS/MS
- Sample Comparison via LC MS/MS
- C terminal via LC MS/MS
- other

### **Objective:**

List any details pertinent to this sample submission.

### **Sequence if known :**

Also email known sequence to: hmf@harvard.edu

### **History :**

Please indicate if you are new to our lab, or if you have submitted previously.

For Lab Use Only: observed staining \_\_\_\_\_ surface area \_\_\_\_\_ estimated weight \_\_\_\_\_ estimated pmole \_\_\_\_\_ Digestion volume: \_\_\_\_\_ Buffer \_\_\_\_\_  
Enzyme \_\_\_\_\_ E/S ratio: \_\_\_\_\_ Digestion Time: \_\_\_\_\_ Ready Volume \_\_\_\_\_ Ready date \_\_\_\_\_ Percent of original sample used \_\_\_\_\_

# PROTEIN SEQUENCE ANALYSIS

HARVARD MICROCHEMISTRY FACILITY

SAMPLE NAME: \_\_\_\_\_  
(This should match tube)

DATE SUBMITTED: \_\_\_\_\_

PHONE(s): \_\_\_\_\_

YOUR NAME: \_\_\_\_\_

FAX: \_\_\_\_\_

**Billing Information:**

PRINCIPAL INVESTIGATOR: \_\_\_\_\_

Institution: \_\_\_\_\_

Billing Address: \_\_\_\_\_

Purchasing Agent Phone #: \_\_\_\_\_

Harvard/Affiliate's 33-digit Account #: \_\_\_\_\_

Non-Harvard User's P.O. Number: \_\_\_\_\_

**3/4 ➤ Billing and Sample Information must be completed before submission of the sample.** Samples should be provided salt, buffer and detergent free. Any other conditions should be discussed before submitting sample. This form is to be used for direct sequencing of samples only and not for samples which are to be digested/separated by the facility prior to sequencing. **Unless otherwise discussed, 10% of the sample is routinely taken for quantitation by amino acid analysis prior to protein sequencing. Please complete an amino acid analysis form to accompany this form.**

**3/4 ➤ Please complete all fields, blank information on this form will delay analysis of your sample!**

MOLECULAR WEIGHT: \_\_\_\_\_ Kd

ESTIMATED WEIGHT: \_\_\_\_\_  $\mu$ g

VOLUME: \_\_\_\_\_  $\mu$ l      BUFFER: \_\_\_\_\_

ESTIMATED AMOUNT: \_\_\_\_\_ pmol

ORGANISM, TISSUE SOURCE: \_\_\_\_\_

ESTIMATED BY: \_\_\_\_\_

BLOTS ONLY: PVDF BRAND, NAME: \_\_\_\_\_

TOTAL SURFACE AREA: \_\_\_\_\_ mm<sup>2</sup>

**3/4 ➤ NUMBER OF CYCLES TO RUN:** Please indicate ONE of the following:

\_\_\_\_\_ Cycles exactly

\_\_\_\_\_ 5 cycles only (minimum)

\_\_\_\_\_ As many as possible\*, specify maximum: \_\_\_\_\_

*\*Note: charge is per cycle run, not data obtained*

**3/4 ➤ Please comment on the purpose of the sequencing and review any special instructions. N.B.:** In the absence of any instructions, 100% of the sample remaining after quantitation by AAA will be subjected to direct amino terminal sequencing.

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**3/4 ➤ Radiolabelled samples are restricted to less than 2000 DPM of <sup>3</sup>H or <sup>14</sup>C.** Such samples will not be accepted without prior clearance with the facility, proper yellow radiolabel tape on the tube, and a printout of the measured DPM.  
Isotope: \_\_\_\_\_ DPM: \_\_\_\_\_

# MASS SPECTROMETRIC ANALYSIS

HARVARD MICROCHEMISTRY FACILITY

DATE SUBMITTED: \_\_\_\_\_

PHONE: \_\_\_\_\_

YOUR NAME: \_\_\_\_\_

FAX: \_\_\_\_\_

**Billing Information:**

PRINCIPAL INVESTIGATOR: \_\_\_\_\_

Institution: \_\_\_\_\_

Billing Address: \_\_\_\_\_

Purchasing Agent Phone #: \_\_\_\_\_

Harvard/Affiliate's 33-digit Account #: \_\_\_\_\_

Non-Harvard User's P.O. Number: \_\_\_\_\_

$\frac{3}{4}$  ➤ **Billing and Sample Information must be completed before submission of the sample.** Incomplete forms will delay your analysis. Samples should be provided salt, buffer and detergent free. **Any other conditions should be discussed.**

$\frac{3}{4}$  ➤ **Note: a 10% aliquot will be taken for amino acid analysis, unless otherwise discussed with W. Lane.** Radiolabeled samples are not accepted without prior permission, and should be properly labeled on this form and on the sample tube.

$\frac{3}{4}$  ➤ Please comment on the purpose of the analysis, (e.g. confirmation of known MW, screening of a protein digest, synthetic peptide purity, post-translational modification, screening for heterogeneity, etc.):

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

	ESI-MS or MALDI-MS?	Sample Name <i>(on tube)</i>	MW <i>(known or estimate)</i>	Weight $\mu$ g	Amt pmol	Vol $\mu$ l	Organism and Tissue Source, Solvent, Comments
1							
2							
3							
4							
5							
6							
7							
8							

MICROCHEMISTRY & PROTEOMICS ANALYSIS FACILITY  
HARVARD UNIVERSITY

William S. Lane, Director  
52 Oxford Street B247  
Cambridge, Massachusetts 02138



Phone: (617) 495-4043  
Fax: (617) 495-1374  
<http://mcb.harvard.edu/microchem>

## Preparation for *In Gel* Proteolytic Digestion and High Sensitivity Technologies

→ **The protocol guidelines below are not a substitute for a discussion of your project. Please do not distribute this to anyone—even your colleagues or benchmates. Always call to discuss the specifics of your project before submitting a sample. Successful projects stem from a thorough understanding by both the researcher and our laboratory of goals, expectations and requirements.**

- **Commit as much protein as is possible**

*Regardless of how sensitive our technologies are, always prep as much protein as possible. DO NOT SKIMP. If it only takes a week to generate a "one-X prep" then spend two weeks and prep 2X. All sequencing technologies, MS or Edman, are mole-based not mass based: e.g. for equal staining a 200K protein has 10X less than a 20K, of course.*

- **Optimize your gel conditions so that you get the greatest amount of protein per gel volume**

*Density (protein to gel volume ratio) matters. There is no restriction on the number of lanes that you send, as long as you have saturated and focused your protein in each lane. Large quantities of protein in diffuse gel volumes can fail; similarly, the way to make small amounts of protein succeed at state-of-the-art levels is to do everything you can to focus that amount in the least gel volume. (Also, note this in excising your bands below.)*

- **Standard SDS-PAGE or 2D gel**

*Run a standard gel that optimizes for the above conditions. Always using the highest quality and fresh reagents/solvents throughout all of these procedures. Reducing, non-reducing, native or gradient conditions are OK as long as the amount of protein has been maximized and the amount of gel minimized. Please contact us if special or unusual conditions or reagents are used and note these on your form.*

- **A gel thickness of 1.0 millimeter is preferred**

*There is evidence that overall recovery maximizes in a gel of 1 mm thickness. Gels of 0.5mm thickness can have greater loss of protein during the stain and destain processes.*

- **Stain with Coomassie Blue, standard conditions, for the minimum time to detect the protein, typically 15 - 30 min.**

*Please remember that the only function of stain in this experiment is to observe your band. Therefore, only stain long enough to accomplish this goal. If your protein requires five hours to visualize then so be it. However, do not stain for 5 hours if your protein is visualized in 15 min. General recipes can be used--contact us only if you need to use a very non-standard condition or reagent.*

- **Destain thoroughly, standard conditions, to a clear background and nicely visualized bands**

*Coomassie and other stains are interfering artifacts in our technologies. Destain long enough to clear your background and still have nicely visualized bands. All excess stain should be removed.*

- **Excise bands tightly, no excess unstained or partially stained gel**

*To maximize the protein to gel volume ratio, excise only the hearts of the bands. Do not include any unstained gel. For example, adding an extra 0.5mm of gel around a 1 x 10mm average band doubles its area, yet the amount of protein gained may be < 5%.*

- **Pool all lanes or spots from a given protein in a single tube**

*One tube per protein, even if there are multiple lanes or spots. DO NOT SPLIT protein bands into multiple tubes. Individual tubes are considered as different samples and will be processed and charged separately.*

- **The tube(s) should be 1.5ml, Eppendorf-style, and in particular: plain.**

*Use plain tubes. There are three items to avoid: 1) no colored tubes, 2) no O-rings and 3) no chemical treatment.*

- Also excise an equivalent area of the same gel that represents your background**  
*This should be from the same gel, processed identically to your samples. This is a blank gel to control for chemical noise and generalized, nonspecific protein background. We only need one control for each 3 to 4 proteins that are submitted.*
- Wash the gel slices in the tube 2 times with 50% acetonitrile in water.**  
*Each wash consists of 0.5 - 1.0 ml of 50% HPLC grade acetonitrile/water for 2 - 3 min. with gentle shaking, discarding the supernatant after each wash. This step is to normalize your gel slices for storage and shipment.*
- After discarding the supernatant from the second wash, you should be left with moist gel slices or spots that are *not* submerged or swimming in any excess liquid.**  
*Do your best to remove all excess liquid. A drop or two is fine. The gel slices remain moist.*
- Close the cap on the tube. Do not use parafilm.**  
*Parafilm can introduce chemical noise into the system. Be careful of introducing dust and surface material to the cap or lip of tube.*
- Freeze at -20 °C or below and you can store that way indefinitely until sent to us.**  
*Any temperature -20 °C or below is fine. Samples have been successfully processed a year later. For that reason, always identically prepare other bands from the same gel at the same time, even if you are not planning to send them. This will allow you to revisit them if they become significant at a later time.*
- Send samples to us on dry ice.**  
*If your shipment is international, it is your responsibility to clear all customs issues before sending. Our address is on the forms.*
- Fill out one Digestion/Separation form for each tube that you are sending to us, including the control.**  
*The form is your lifeline to each sample. Do not leave anything blank. We encourage you to briefly outline your goals and the biochemical significance in the instruction area. If there is information you have that is relevant to interpretation of the analysis or handling of your sample, let us know on the form! The Digestion/Separation form is the only form to fill out, but one is required for each tube, including control. Do NOT fill out a Protein Sequence form or Mass Spectrometry form.*
- Understand keratin contamination:**  
*High sensitivity analysis from gels comes with a price: keratin contamination is always observed. Wearing gloves is not enough. In fact, the typical contamination is not your own hands but rather non-specific dust contamination introduced in the steps after one has run the gel. Observed keratins do not necessarily have to be at the same MW as your band. Note: you cannot get rid of it but you can minimize it. Rinse all surfaces while you work (simple rinses with HPLC grade water) that contact the gel from the point that you take the gel apart to the final storage. These surfaces include the outside of your gloves, staining trays, scalpels, razor blades, tongs and the inside of the final 1.5ml tubes. Note bene: the less protein you have the more significant the non-specific keratin background will be to successful analysis of your sample. Regardless what organism you are working with, do not be surprised when keratin is observed.*
- Time**

Login ("day 0"):	<i>Note: most delays are due to incomplete forms and billing information.</i>
Week 1 (day 4 - 9):	<i>In gel reduction, alkylation and proteolytic digest of 100% of the sample(s).</i>
Week 2 (day 7 -15):	<i>High sensitivity LC/MS of ~10% of the digest mixture. Purpose: Ascertain whether digest was successful. A fax will be sent that simply indicates whether we are proceeding or not. There is no detailed data analysis at this point and usually no need for a discussion.</i>
Week 3 (day 10 - 24):	<i>In depth review of acquired MS/MS spectra before consuming the remaining 90% of the digest. MS/MS sequencing by MS/MS correlation analysis and reporting.</i>
Week 4 (day 14 - 30):	<i>Discussion of project results. Further HPLC analyses and single sequence attempts, if necessary.</i>
- Alternative stains.**  
*Sypro Ruby, copper, zinc and modified silver stains are compatible with the downstream technologies with certain caveats. Please discuss with us before using. Reading the literature is not a substitute for a thorough discussion with us. Remember the obvious: a more sensitive stain does not give you more protein!*