Instruction for Sample Preparation and Submission

1. Please fill out the <u>Sample Submission Form for Protein MS/MS Identification & Analysis</u> or <u>Sample Submission Form for Quantitative Proteome Analysis (iTRAQ)</u>, have the PI approved and signed.

2. Protein samples in solution or in excised spots/bands from gels can be submitted for protease (trypsin or Lys-C) digestion and LC-MS/MS analysis for protein identification.   You can also do protease digestion by yourself using enzymes and reagents prepared by the Core Lab with some charges.

3. Radioactive and biohazard samples CANNOT be accepted.

4. Stain gels with Coomassie, Sypro Ruby, or MS compatible silver staining method. Coomassie and Sypro Ruby staining methods are preferred.

5. Try to minimize the detergent (e.g. SDS) and salt concentration as much as you can.

6. Reconstitute the pre-digested peptides in 0.1% formic acid solution for an appropriate LC-MS/MS analysis.

7. For the LC-MS/MS protein identification, the least protein amount should be ~10 µg (crude extracts), ~10 fmol (relatively purified) in ~10-20 μl, or ~100 fmol in excised gel spots.   For the phosphorylation site analysis, a higher protein amount will be needed depending on the portion of the phosphorylated protein.

8. For quantitative proteome analysis (iTRAQ), ~100 µg proteins for each samples are required.

9. For the phosphorylation site analysis, a MOAC (metal oxide affinity chromatography) on TiO_2 beads will be used for the phosphopeptide enrichment prior to the LC-MS/MS analysis.

10. For the LC-MS/MS analysis a nanoLC system with a C18 reverse phase column (Acclaim PepMap® RSLC, 75 μm ID x 250 mm, 2 µm, 100Å) is used to separate peptides.   A flow rate of 300 nl/min is employed with a linear gradient from 5 to 30% acetonitrile containing 0.1% formic acid over 40 - 180 min.   For proteins of low complexity, e.g. proteins from 2-D gel spots or purified proteins, we recommend a 40-min LC separation.   For protein mixtures of moderate complexity, e.g. protein bands from 1-D gel, we recommend a 90-min LC separation.

11. If your favorite protein is not present in the publicly-available database, please provide us your favorite protein sequence used for protein identification.

12. Submit the complete form with your sample(s) to A227, Agricultural Technology Building, Academia Sinica.

13. If you have any other questions, please contact personnel in the Core Lab.

14. Please acknowledge the effort performed at this Core Lab in the Acknowledgement of your manuscript or share a co-authorship when you prepare for publication.　Thank you!