
Nevada Proteomics Center

Protein Digestion and Mass Spectrometry Materials and Methods

Protein digestion and mass spectrometry.

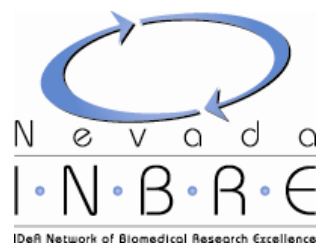
Spots are digested using Investigator™ Proprep™ (Genomic Solutions, Ann Arbor; MI), using a previously described protocol (Rosenfeld, J., et al. Analytical Biochemistry (1992) 203. pp173-179) with some modifications. Samples are washed twice with 25mM ammonium Bicarbonate (ABC) and 100% acetonitrile, reduced and alkylated using 10mM DTT and 100mM Iodoacetamide and incubated with 75ng Trypsin in 25mM ABC for 6 hrs at 37o C.

Samples are prepared and spotted onto a MALDI (Matrix Assisted laser Desorption Ionization) target with ZipTipu-C18 (Millipore, Billerica; MA). Samples are aspirated and dispensed 3 times and eluted with 70% ACN, 0.2% formic acid and overlaid with 0.5µl 5mg/ml MALDI matrix (α-Cyano-4-hydroxycinnamic acid) and 10mM ammonium phosphate.

All mass spectrometric data was collected using an ABI 4700 MALDI TOF/TOF (Applied Biosystems, Foster City; CA). The data was acquired in reflector mode from a mass range of 700 – 4000 Daltons and 1250 laser shots were averaged for each mass spectrum. Each sample was internally calibrated on trypsin's autolysis peaks. The eight most intense ions from the MS analysis, which were not on the exclusion list, were subjected to MS/MS. For MS/MS analysis the mass range was 70 to precursor ion with a precursor window of -1 to 3 Daltons with an average 5000 laser shots for each spectrum. The data were stored in an Oracle database.

The data was extracted from the Oracle database and a peak list was created by GPS Explorer software (Applied Biosystems, Foster City; CA) from the raw data generated from the ABI 4700. This peak list was based on signal to noise filtering and an exclusion list and included de-isotoping. The resulting file was then searched by Mascot (Matrix Science, Boston; MA). A tolerance of 20ppm was used if the sample was internally calibrated and 200 ppm tolerance if the default calibration was applied. Database search parameters include 1 missed cleavage, oxidation of methionines and carbamidomethylation of cysteines.

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