

MALDI-TOF MS Analysis of Protein Tryptic Digestion for Protein ID (Code C):

1. General Procedure

A saturated α -cyano-4-hydroxycinnamic acid in 70% acetonitrile/0.1% trifluoroacetic acid is used as the matrix solution. 1 μ l of protein tryptic digestion mixture is spotted on the sample target, and then 1 μ l of saturated matrix solution is added on top. After the crystal is formed, the sample target is inserted into the mass spectrometer.

MALDI MS is acquired on Voyager-DE STR MALDI-TOF mass spectrometer (Applied Biosystems). The instrument is equipped with a 337 nm laser. The mass spectrometric analysis is performed with reflectron mode at positive. Acceleration voltage is set at 20 kV, grid voltage at 72%, guide wire at 0.001%, and delay time at 190 nsec. The mass spectra are externally calibrated by the molecular weights of a mixture of standard peptides, or internally calibrated by trypsin autolysis peaks.

2. Mass Accuracy

The mass accuracy is 0.01% when calibrated internally, while the accuracy is 0.05% when calibrated externally.

3. How to Read the Results?

A peak list of m7 December, 2007 search for protein ID yourself. The peak list can be copied and pasted into the database search software. The m/z value in the spectrum is the peptide mass plus 1 (M+H)+.

A list of web sites for database searching based on peptide mass fingerprinting:

Mascot: http://www.matrixscience.com/cgi/search_form.pl?FORMVER=2&SEARCH=PMF

ProFound: http://prowl.rockefeller.edu/profound_bin/WebProFound.exe

MS-Fit: <http://prospector.ucsf.edu/ucsfhtml4.0/msfit.htm>