**de novo** Sequence Analysis of Internal Protein Fragment with ORFinder-N Mass Sequencing Kit

The ORFinder-N Mass Sequencing Kit is used for isolating protein N-terminal fragments as sulfonic acid derivatives from trypsin digest mixtures. The isolated N-terminal fragment possesses a strong negative charge, while the C-terminal fragment possesses a strong positive charge due to the existence of either an arginine or homoarginine residue. Therefore, for the product ion in MALDI-PSD (MS/MS) measurement, the ions included on the N-terminal side containing the negatively charged sulfonic acid moiety (a, b, c ions) are suppressed, while the ions included in the C-terminal side (x, y, z ions) are promoted. Generally, since the y ion is predominantly detected among the ions derived from C-terminal fragmentation, it is far simpler to interpret the product ion in the PSD (MS/MS) spectrum. This selective promotion of the y ion series enables direct identification of the amino acid sequence from the spectrum. This article introduces an example of de novo sequence analysis of an internal protein fragment using the AXIMA®-CFR Plus, in which the ORFinder- Mass Sequencing Kit protocol was utilized to derivatize fragments resulting from a protein trypsin digest mixture to the N-terminal sulfonic acid forms. Fig. 1 shows the scheme of the protein internal fragment de novo sequence analysis using the ORFinder- Mass Sequencing Kit.

**Fig.1** Protein Internal Fragment de novo Sequence Analysis using ORFinder- Mass Sequencing Kit

Spots separated by 2D electrophoresis of E. Coli extract were processed according to the ORFinder- Mass Sequencing Kit internal fragment analysis protocol.
The y ions are predominantly detected in the respective MALDI-PSD spectra, Thr-Ala-Xle-Xle-Asx-His-Xle-Asp-Thr-Met (Tyr)*-Ala-Glu-Arg and Gly-Ala-Asn-Phe-Xle-Ala-Val-His-Glu-Met (Tyr)*-Xle-Asp-Gly-Phe-Arg sequences were identified directly from the spectra, and were confirmed as internal fragments of a DNA Protection Protein During Starvation via BLAST search.

*Met is converted to Met (O2) (Δm/z 163.03) during the biotin reagent sulfonic acid derivatization process, and is not distinguishable from Try (Δm/z 163.07).