

Trypsin NB Premium Grade, MS approved

A New Trypsin Peptide (TP) Standard For Internal Calibration

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Introduction

SERVA Trypsin NB Premium Grade has been developed especially for proteomic investigations. It is ideally adapted to digest proteins prior to mass spectrometric analysis. Based on excellent and proprietary production procedures, trypsin product exhibit exceptionally low autocatalytic activity resulting in unique stability. Nearly any trypsin peptides can be detected in the reaction mixture after standard digestion of single proteins or complex protein mixtures.

To meet requirements where standard peptides are needed for internal calibration SERVA has now developed TP Standard containing trypsin to generate masses m/z 842 and 2211. TP Standard facilitates easy internal calibration to enhance mass accuracy in MS analysis and can be adjusted to any experimental conditions. TP Standard will be delivered with the Trypsin NB products (Trypsin NB Sequencing Grade, modified, cat. no. 37283.01; Trypsin NB Premium Grade, MS approved, cat. no. 37284.01).

Methods

Bovine serum albumin (BSA) was used as test protein and 300 ng were separated by gel electrophoresis and in-gel digested with 10 ng/ul trypsin in 25 mM NH_4HCO_3 . TP Standard was added in different ratios (Trypsin : TP Standard) to the digestion mix which was incubated at 37 °C over night. The generated peptides were extracted from the gel and analysed by MALDI-TOF MS in reflectron mode using the Bruker Ultraflex I mass spectrometer. Protein identification was done with the Mascot search engine (Matrix Science, UK).

Results

SERVA Trypsin NB Premium Grade was compared to Promega Trypsin Gold (Fig. 1) using BSA as standard protein. With Promega enzyme a Mascot score of 156 was identified. A significantly higher score of 165 was achieved for SERVA Trypsin. Additionally, no trypsin specific peptides were determined using SERVA Trypsin indicating a highly reduced autocatalytic activity of this product. In contrast Promega Trypsin possessed significant autodigestion and typical trypsin peptides were identified in the MS spectrum.

In some applications trypsin masses are helpful for additional internal calibration of protein digests to enhance mass accuracy. To facilitate the use of SERVA Trypsins for these experiments the TP Standard was developed. TP Standard is easily added to the SERVA Trypsin and desired peptides of m/z 842 and 2211 are obtained after digestion (Fig. 2). The amount of TP Standard can be adjusted to the desired experimental conditions. Fig. 2 shows several ratios. In the experiments performed using BSA as test protein, a combination of 1:5 of TP Standard to SERVA Trypsin NB Premium Grade was sufficient for significant peaks that can be used for internal calibration (Fig. 3). Signal to noise ratios of the TP Standard proteins ranged from 14 to 95. Similar results were obtained for ovalbumin and overexpressed C3 exoenzyme as test protein (data not shown).

In all digested samples shown herein, similar high Mascot scores were determined after data analysis. Using the TP Standard and additional internal calibration after standard external calibration, mass accuracy increased slightly and therewith the obtained Mascot score increased from 167 to 183 for BSA identification.

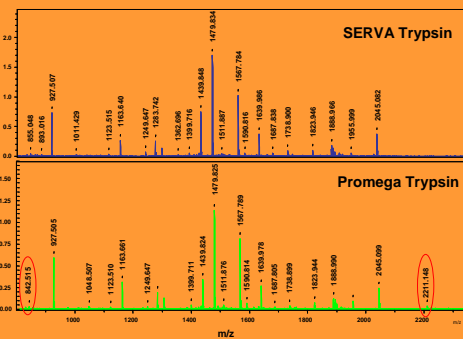


Fig. 1: Comparison of SERVA Trypsin NB Premium Grade and Promega Trypsin Gold. Spectra of BSA digested with SERVA Trypsin NB Premium Grade, MS approved and Promega Trypsin Gold, Mass Spectrometry Grade are shown. Trypsin masses are indicated.

Data were kindly provided by



and are presented by

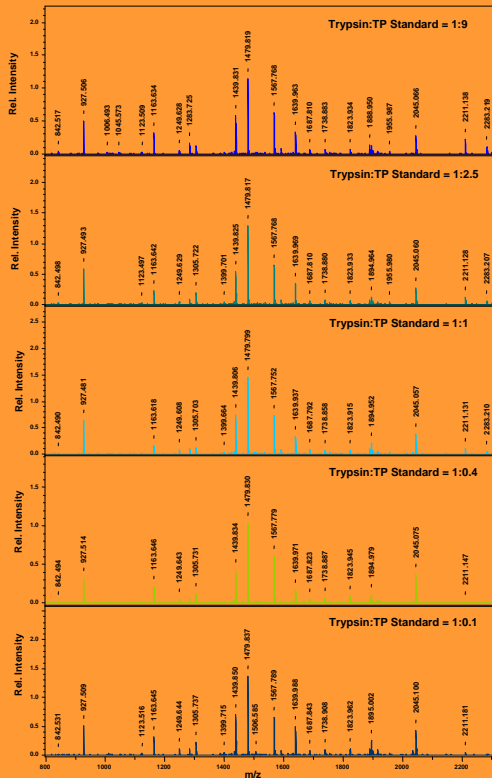


Fig. 2: Spectra of a BSA digestion using SERVA Trypsin NB Premium Grade and TP Standard in varying amounts. BSA was in-gel digested and SERVA Trypsin NB Premium Grade, MS approved was mixed with TP Standard in ratios of 1:9, 1:2.5, 1:1, 1:0.4, 1:0.1.

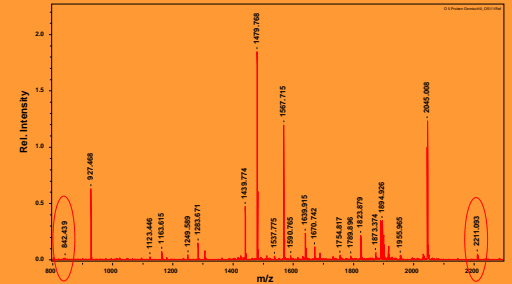


Fig. 3: Peptide mass fingerprint of BSA using SERVA Trypsin NB Premium Grade and TP Standard in a ratio of 5:1. BSA digestion was carried out with SERVA Trypsin NB Premium Grade, MS approved and TP Standard. Desired masses of m/z 842 and 2211 are indicated.

Conclusions

SERVA Trypsin NB Premium Grade, MS approved is a high quality trypsin generating excellent peptide mass fingerprints in standard MS-based protein identification. SERVA Trypsin NB Premium Grade provided significantly better Mascot identification scores than Promega Trypsin Gold under all conditions tested.

Addition of TP Standard to SERVA Trypsin NB products allows for additional internal calibration. TP Standard has to be added to the trypsin solution in a ratio of 1:5. Using TP Standard and additional internal calibration, mass accuracy and therewith Mascot scores increases after MALDI-MS measurement and Mascot data base search for protein identification.

Thus, in any application where internal calibration is needed TP Standard is an ideal solution for generation of high quality peptide mass fingerprints to identify proteins by MS analysis.