proteomics

Trypsin In-Gel Digest Kit: A Complete Kit for the Digestion of Proteins from SDS-PAGE Gels

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Application Notes

- Compatible with Coomassie®, SYPRO® Orange, SYPRO Ruby and ProteoSilver™ stained gels
- Resulting samples are suitable for analysis by MALDI-MS or HPLC-MS
- Includes highly purified proteomics-grade Trypsin that displays only minimal autolytic activity and no chymotryptic activity, which simplifies resulting peptide analysis

Introduction

In-gel tryptic digests of 1-dimensional (1-D) or 2-dimensional (2-D) acrylamide gels are commonly used in proteomics to identify unknown proteins of interest. The unknown proteins are excised from the gel, digested with trypsin then analyzed via MALDI-MS or HPLC-MS with subsequent database searching. The ProteoProfile™ Trypsin In-Gel Digest Kit (Product Code PP 0100) is a complete kit that contains everything needed to perform a trypsin in-gel digest. The kit contains proteomics-grade trypsin, the most important component of the kit, that has been chemically modified through reductive methylation to reduce autolysis and minimize trypsin autolytic fragments. The lysine residues are dimethylated following reductive methylation protecting the trypsin against autodigestion. The enzyme is then TPCK treated to remove any chymotrypsin activity, then further purified by affinity chromatography yielding a highly purified trypsin suitable for proteomics work.

Optimized destaining, proven performance

The ProteoProfile Trypsin In-Gel Digest Kit has use-tested procedures and reagents for polyacrylamide gels stained with Coomassie® Brillaint Blue or SYPRO® Orange or Ruby dyes (Figure 1). The destaining step has been optimized to destain faster than competitors and published methods. If silver stained gels are used, a different destaining step is required (i.e., ProteoSilver™ Plus Silver Staining Kit, Product Code PROT-SIL2). A destaining solution for silver stained gels is included in the ProteoSilver Plus Kit and the gel pieces should be destained according to the included protocol.

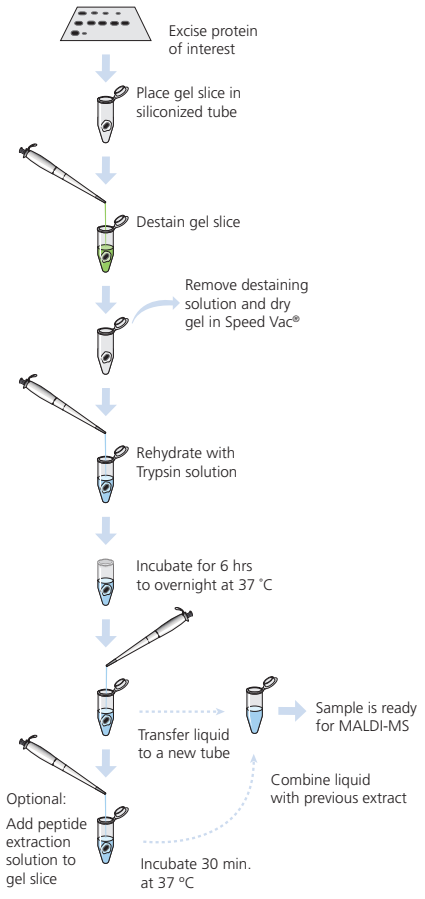

Figure 1. Overview of ProteoProfile Trypsin In-Gel Digest Kit procedure.

To illustrate the performance of the ProteoProfile Trypsin In-Gel Digest Kit, an unknown protein from a total protein extract was identified. Lyophilized *E. coli* was extracted with Cellular and Organelle Membrane Solubilizing Reagent (Product Code C 0356) to yield a 2.5 mg/ml protein solution. This extract was reduced and alkylated then separated by 2-dimensional electrophoresis. A protein spot was randomly chosen, excised, and digested with trypsin using the ProteoProfile Trypsin In-Gel Digest Kit (Figure 2). The digested peptides were analyzed by MALDI-MS followed by database searching (Figure 3). The chosen protein spot was identified as outer membrane protein 3a from *E. coli* with 30% sequence coverage (Table 1).

Figure 2. *A solution of 2.5 mg/ml E. coli in Cellular and Organelle Membrane Solubilizing Reagent of ProteoPrep™ Total Extraction Sample Kit (Product Code PROT-TOT) was sonicated, then reduced with tributyl phosphine and alkylated with iodoacetamide (Product Code PROT-RA). 109 µg of protein was loaded on a 7 cm, pH 4-7 IPG strip (Product Code I 2906), focused for 50,000 volt hours, then run on a 4-20% Tris-Glycine SDS-PAGE gel at 150 volts for 70 min. The gel was then stained with EZBlue Gel Staining Reagent. A random spot was then excised and tryptically digested using the ProteoProfile Trypsin In-Gel Digest Kit.*

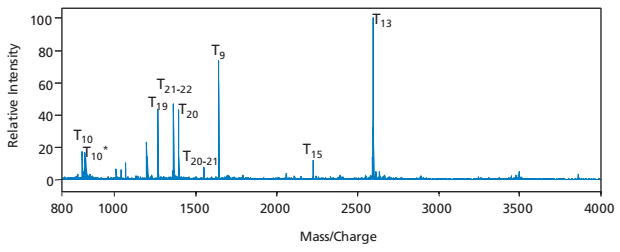

Figure 3. The sample was desalted using a ZipTip™ C18 pipette tip from Millipore and eluted directly onto the MALDI target using the MALDI matrix (α-cyano-4-hydroxycinnamic acid, 10 mg/ml in 70% ACN, 0.03% TFA). MALDI analysis was performed in the reflection positive ion mode.

Table 1. The resulting monoisotopic masses were searched against the NCBI database at a tolerance of 150 ppm. The protein was identified as outer membrane protein 3a from E. coli with the matched peptides providing 30% sequence coverage.

Tryptic Fragment	Amino Acid	M+H⁺
T_9	104-117	1654.8328
T_{10}	118-124	818.4347
T_{10}*	118-124 oxidized	834.4296
T_{13}	135-159	2601.2949
T_{15}	191-213	2232.1665
T_{19}	252-263	1280.6487
T_{20}	264-276	1409.6661

Missing Cleavages	Amino Acid	M+H⁺
T_{20-21}	264-277	1565.7572
T_{21-22}	277-288	1378.7694

Highly active and stable trypsin

The modified trypsin is highly active and stable. The digestion times can be modified to suit researchers' needs. To demonstrate the flexibility of digestion, carbonic anhydrase II used as a model protein, underwent tryptic digestion from 2 to 24 hours to determine the length of time necessary for complete digestion. A total of 2 µg of carbonic anhydrase II per lane was run on a 1-D SDS-PAGE gel in multiple lanes and stained with EZBlue™ Gel Staining Reagent (Product Code G 1041). The gel slices were excised, destained and tryptically digested with the ProteoProfile Trypsin In-Gel Digest Kit for varying lengths of time. After the appropriate time, the digestion was stopped with the addition of TFA. It was found that carbonic anhydrase II was almost completely digested after 2 hours (Figure 4). After four hours, the digestion was complete. Digestion time will change with protein size and number of tryptic cleavages, but using carbonic anhydrase II, four hours was sufficient for complete digestion.

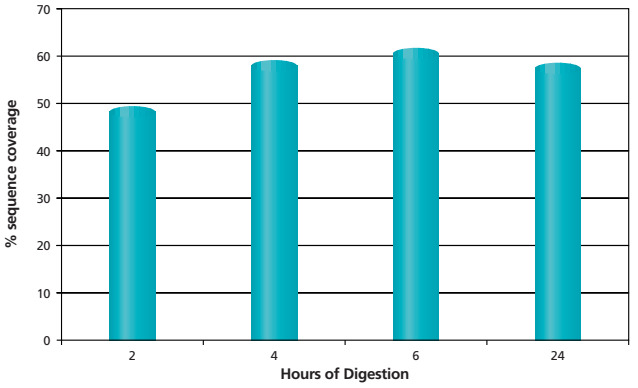

Figure 4. Sequence Coverage vs. Digestion Time. *Multiple lanes containing 2 µg of reduced and alkylated carbonic anhydrase II were separated on a 4-20% Tris-Glycine gel and stained with EZBlue Gel Staining Reagent. The protein bands were excised then tryptically digested using the ProteoProfile Trypsin In-Gel Digest Kit. Separate digests were set up for 2-, 4-, 6- and 24-hour time digests. At the appropriate time, the digest was stopped with the addition of TFA. The digested peptides were then analyzed by MALDI-MS.*

The modified trypsin is also very stable once reconstituted. Reconstituted trypsin stability was tested by reconstituting three trypsin vials according to the kit protocol (in HCl followed by ammonium bicarbonate). The reconstituted vials were stored at 4 °C for up to 7.5 weeks. To test the trypsin stability, 2 µg of carbonic anhydrase II was separated on a 4-20% gel stained with EZBlue Gel Staining Reagent (data not shown). The protein bands were excised and tryptically digested using the ProteoProfile Trypsin In-Gel Digest Kit. The reconstituted trypsin, which was used, had been stored at 4 °C for various lengths of times. After digestion, the peptides were analyzed by MALDI-MS with subsequent database searching. The results indicate that the reconstituted trypsin is still active after being stored at 4 °C for 7.5 weeks.

Quick and easy method

The ProteoProfile Trypsin In-Gel Digest Kit is a complete kit containing all necessary reagents to perform a trypsin in-gel digest. The kit also includes proteomics-grade trypsin that has been shown here to be stable after being stored at 4 °C for up to 7.5 weeks. The ProteoProfile Trypsin In-Gel Digest Kit provides a quick and easy method for the digestion of up to 100 samples.

Ordering Information

Product	Description	Unit
PP 0100	ProteoProfile™ Trypsin In-Gel Digest Kit	1 kit

Kit Components

Proteomics Grade Trypsin
Destaining Solution
Trypsin Reaction Buffer
Biotech Grade Acetonitrile
Trypsin Solubilization Reagent
Peptide Extraction Solution