3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Trypsin Singles, Proteomics Grade

Catalog Number **T7575**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
The Trypsin Singles, Proteomics Grade Kit provides all necessary reagents for tryptic digestion of a protein sample. Trypsin Singles are conveniently packaged in 96 × 1 µg vials for one time use, eliminating the need to aliquot, decreasing the chance for cross-contamination, and retaining maximum enzymatic activity.

Trypsin is a pancreatic serine endoprotease. The Trypsin Singles, Proteomics Grade, Enzyme exhibits excellent proteolytic efficiency and generates more tryptic peptides than standard trypsins. The trypsin has been extensively purified from porcine pancreas. This purification, along with the reductive methylation of the lysine residues, produces a stable product that is resistant to autolysis.[1] Chymotryptic activity of the enzyme is removed through TPCK treatment. The product is further purified by affinity chromatography and dried from dilute acetic acid. This process yields a highly purified trypsin product that is suitable for proteomics work. Highly purified and chemically stabilized trypsin offers excellent performance for use in either solution or in-gel tryptic digestions.

Trypsin is a highly specific protease that cleaves proteins or peptides on the carboxyl side of arginine (R) or lysine (K) residues. The rate of hydrolysis is slower when an acidic residue is located on either side of the cleavage site and cleavage may not occur if a proline residue is on the carboxyl side.[2-6] The enzyme also exhibits esterase and amidase activities.[2]

Due to its highly specific cleavage, trypsin is routinely used in proteomics for peptide mapping and protein sequencing.[2-6] Trypsin is a highly useful proteolytic enzyme for mass spectrometry. Following digestion of a protein with trypsin, each proteolytic fragment contains a basic arginine (R) or lysine (K) residue.

Each fragment is, therefore, amenable for mass spectrometric analysis in positive ion mode, because positively charged ions “fly well” in mass spectrometers. Mass spectrometry may be used to analyze the digested protein mixture (without prior separation) to generate a mass spectrum. The molecular masses of all peptide fragments within the mass spectrum are then determined using appropriate software. The mass spectrum combined with the molecular mass data comprises a Peptide Mass Fingerprint (PMF) - this being a unique pattern for any given protein. The derived peak list is then submitted to a search engine, where it is compared to archived lists. In this way protein identification can readily be obtained. For more detailed mass spectrometric analysis, tandem mass spectrometry (*i.e.*, MS/MS) may be used.

Average Molecular Mass: 23.29 kDa

Optimal pH:[2] ~8.0

Specific Activity: ≥10,000 BAEE units per mg of protein.

Components
- Trypsin Singles, Proteomics Grade, Enzyme (Catalog Number T7200)
- Trypsin Solubilization Reagent (Catalog Number T2073)
- Trypsin Reaction Buffer (Catalog Number R3527)
- Biotech Grade Acetonitrile (Catalog Number 494445)

Equipment and Reagents Required But Not Provided
- Ultrapure water (18 MΩ·cm or equivalent)
- 37 °C heating block or heating bath.
- ProteoPrep® Reduction & Alkylation Kit (Catalog Number PROTRA)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Ultrapure water (18 MΩ·cm or equivalent) is recommended for preparation of all reagents.

Trypsin Solubilization Reagent – this reagent contains 1 mM HCl and is supplied ready-to-use.

Trypsin Reaction Buffer – add 10 ml of water and 1 ml of Biotech Grade Acetonitrile (Catalog Number 494445) to the bottle. After reconstitution, the bottle contains a solution of 40 mM ammonium bicarbonate and 9% acetonitrile.
Note: The Reaction Buffer does not require pH adjustment (the intrinsic pH is ~8.2).

Trypsin Singles, Proteomics Grade, Enzyme – each vial contains 1 µg of trypsin and is supplied ready-to-use.

Storage/Stability
It is recommended to store the kit at 2–8 °C. All reagents are stable for at least 1 year when stored properly. The Trypsin Reaction Buffer is stable for up to one month after preparation, when stored at 2–8 °C. The Trypsin Singles, Proteomics Grade, Enzyme are intended for one time use.

Procedure
Solution Digestion
The described procedure does not detail the preparation of the protein sample. For peptide or protein digestion, a ratio of between 1:100 and 1:20 (w/w) of enzyme to substrate is recommended.

The specificity and activity of trypsin is retained in systems containing up to 20% organic solvent.[7] In addition, trypsin retains most of its activity in 2.0 M urea, 2.0 M guanidine HCl, or 0.1% SDS.[3-6] The digestion pattern may be influenced by the buffer composition.[8]

A peptide such as the oxidized insulin B chain (Catalog Number I1764) may be run as a control for all experiments.

1. Remove the required number of Trypsin Singles vials (Catalog Number T7200) from the 96 well tray.

2. Separate the vials by carefully cutting the plastic binding between the vials and caps using an appropriate cutting device.

3. Remove the caps from the vials.

4. Add 1 µl of Trypsin Solubilization Reagent (Catalog Number T2073) to each Trypsin Singles vial.

5. Add 50 µl of a reduced and alkylated protein sample, containing no more than 100 µg of protein, to the vial. Ensure the pH of the protein sample is ~8.0 prior to addition to the vial. Vortex the sample to mix.
   Note: Prior reduction and alkylation of the protein sample solution results in a more complete and faster digestion of the protein; thereby, generating higher sequence coverage.

6. Add 49 µl of the prepared Trypsin Reaction Buffer to each vial. The final NH_4HCO_3 buffer concentration will be ~20 mM. Vortex the sample to mix.

7. Incubate the sample at 37 °C to digest the target protein. The recommended incubation time at this temperature is 2–18 hours, depending on the enzyme to substrate ratio. An enzyme to substrate ratio of between 1:20 to 1:100 (w/w) is typical for digestion with trypsin. Lower relative amounts of enzyme require longer digestion times. If MALDI-TOF MS analysis is to be performed at this step, acidification with trifluoroacetic acid (TFA) may be needed prior to matrix addition.

In-Gel Digestion

Trypsin Singles, Proteomics Grade may also be used for in-gel protein digestions with subsequent identification by mass spectrometry. Digestion procedures from gels or on membranes have been published.[9-16] The described procedure is intended for use with a dried and destained gel piece contained within a siliconized microcentrifuge tube or equivalent.

1. Add 5 µl of the Trypsin Solubilization Reagent to one Trypsin Singles vial. Briefly vortex the vial to ensure the trypsin is dissolved.

2. Add 45 µl of the Trypsin Reaction Buffer to the vial and mix. The final trypsin concentration is 20 µg/ml.

3. Add 20 µl (0.4 µg of trypsin) of the prepared Trypsin Single (step 2) to the tube containing the dried gel sample.

4. Following rehydration of the gel slice, add 50 µl of the prepared Trypsin Reaction Buffer to the tube.

5. Confirm that the gel piece is located at the bottom of the tube and covered with liquid. The sample may be briefly centrifuged to collect the solution and the gel slice at the bottom of the microcentrifuge tube.

6. Incubate the sample at 37 °C for 4 hours to overnight.
   Note: A shorter digestion time may suffice, but will likely yield slightly lower sequence coverage.

7. After incubation, remove the liquid from the tube containing the gel piece and transfer this solution to a new tube. This solution contains the extracted tryptic peptides. If MALDI-TOF MS analysis is to be performed at this step, acidification with trifluoroacetic acid (TFA) may be needed prior to matrix addition.

Specificity

An example of the high specificity of Trypsin Singles is illustrated in Figure 1. The HPLC data show the peaks obtained from a solution digest of oxidized insulin B chain (Catalog Number I1764).

The sequence of oxidized insulin B chain is:

FVNQHLC$_{ox}$GSHLVEALYLVC$_{ox}$GER GFFYTPK A

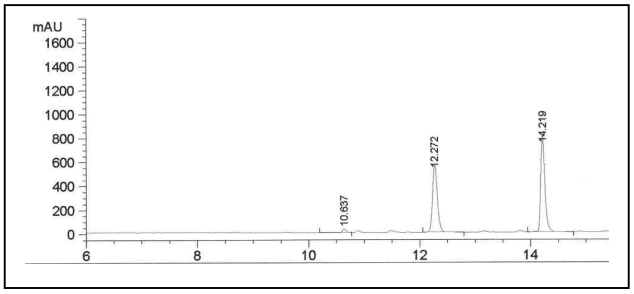
↑ ↑

Tryptic Cleavage Sites

Following an extensive 18 hour digestion, only the expected peptides were generated, with no indication of non-specific proteolytic activity.

Figure 1.

HPLC data (trace) showing the peptides obtained following a trypsin solution digestion of oxidized insulin B chain.



Oxidized Insulin B chain (100 µg) was digested using Trypsin Singles, Proteomics Grade, Enzyme (1 µg) for 18 hours at 37 °C in 100 µl of buffer, pH 8.5. A 10 µg aliquot of the digested substrate was then separated via HPLC using a Supelco Discovery C_{18} column (15 cm × 4.6 mm, 5 micron, Catalog Number 504955), with a 20 minute linear gradient from 5–50% B at 1 ml/min. UV detection was employed at 214 nm. Solvent A was 0.1% (v/v) TFA in water. Solvent B was 0.08% (v/v) TFA in acetonitrile.

References

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