Trypsin, Active
Recombinant swine protein expressed in yeast cells

Catalog # T575-31N
Lot # L2146-8

Product Description
Recombinant tag-free swine trypsin (10-end) was expressed in yeast cells. The Enzyme Commission number is EC 3.4.21.4.

Gene Aliases
Trypsinogen

Formulation
Recombinant protein stored in 10mM HCl pH 3, 20 mM CaCl₂, 30% glycerol.

Digestion Conditions
Catalytic pH range: 7.0 ~ 9.0

Storage and Stability
Store product at –20°C for up to 1 year. Aliquot enzymes to avoid freeze / thaw cycles.

Scientific Background
Trypsin is a serine protease that is produced in the acinar exocrine cells of the pancreas. The enzyme cleaves peptides at the C-terminal side of lysine and arginine amino acid residues. Recombinant pancreatic trypsin is a widely biochemical tool used in processes, which include: recombinant insulin production, cell culture, cell fermentation, protein peptide mapping, proteomic sequencing and cell dissociation. Trypsin function is inhibited by serine protease inhibitors (e.g. TLCK, PMSF), and metal chelating agents (e.g. EDTA).

Purity
The purity of trypsin was determined to be >95% by densitometry, approx MW 21-26 kDa.

Specific Activity
The specific activity of trypsin was determined to be 10,500 BAEE/mg protein.

Sample Data:
HPLC results for digestion of the polypeptide NH₂-YGKRLWK-COOH by Trypsin at an enzyme : substrate mass ratio of 1:1,000 for 10 min at 37°C. The digestion products are YGK, YGKR, R, RLWK and LWK.

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<table>
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<tr>
<th>Catalog #</th>
<th>Aliquot Size</th>
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<tr>
<td>T575-31N-01</td>
<td>1 mg</td>
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FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.
Assay Protocol

Reaction Components

**Active Trypsin Protease (Catalog #: T575-31N)**

Active Trypsin diluted in 1mM HCl (pH 3) to a concentration of 1 µg/µl

**Trypsin Digestion Buffer (User Prepared)**

50mM ammonium bicarbonate, pH 8 or a denaturing buffer such as 50mM Tris-HCl pH 8, containing 8M urea or 0.1% SDS

Solution Digestion Protocol

The following conditions may be different for different proteins. Optimize the protocol for each specific protein.

**Step 1.** Dissolve protein in Trypsin Digestion Buffer

**Step 2.** Add Trypsin to a final protease: protein ratio of 1:20 (w/w), ensuring that the protein concentration is at least 0.1 mg/ml.

(a) Incubate at 37°C for at least 2 hours to overnight depending on the enzyme to protein ratio

(b) Remove an aliquot to determine the extent of digestion by subjecting the aliquot to reverse phase HPLC or SDS-PAGE

(c) Continue incubation at 37°C until the desired digestion is achieved

(d) Optional: add fresh Trypsin if necessary.

**Step 3.** Trypsin digestion can be stopped by freezing or by lowering the pH of the reaction below pH 4 by adding formic, acetic, or trifluoroacetic acid

(a) Trypsin will regain activity above pH 4

(b) Digested samples can be stored at -20°C

Other Uses for Trypsin

- For in-gel digestion, follow standard protocols.
- For cell dissociation reagent and removing adherent cells from a culture surface, prepare 0.025% to 0.5% EDTA (trypsin-EDTA).

Activity Definition (BAEE unit/mg)

SignalChem’s Trypsin activity is defined by the following:

One BAEE unit produces a ΔA<sub>253</sub> of 0.001 per min at pH 7.6 at 25 °C using BAEE as substrate.

Reaction volume = 3.0 ml (1 cm light path).

3.0 BAEE Units = 1 USP Unit