

Immobilized Trypsin, Agarose

Recombinant swine protein expressed in yeast cells

Catalog # T575-31AN

Lot # L2158-6

Product Description

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

Matrix: cross-linked agarose
Average Particle Size: 90 μ m
Ligand Density: 1 mg protein / ml matrix
Maximum Flow Rate, Pressure: 100 cm/h, 0.5 bar

Gene Aliases

Trypsinogen

Storage and Stability

Store product at 2 - 4°C for up to 6 months.

Digestion Conditions

Catalytic pH range: 8.0 ~ 10.0
Catalytic temperature range: 20 ~ 37°C
Enzyme : Substrate Ratio: 1:200 ~ 1:3,000

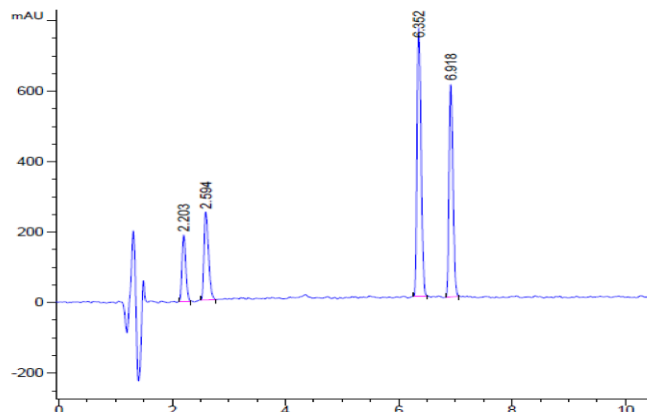
Scientific Background

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).

Immobilized trypsin is covalently cross-linked to an agarose matrix circumventing the need for enzyme removal after cleavage. The resin can be reused 10-20 times after regenerating when proper storage conditions are followed.

Specific Activity

Sample Data



HPLC Results for digestion of the polypeptide NH₂-YGKRLWK-COOH by Trypsin an enzyme : substrate mass ratio of 1:500 for 4 hours. The digestion products are YGK, YGKR, RLWK and LWK.

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Catalog Number	T575-31AN
Lot #	L2158-6
Format	Stored in 50% glycerol
Storage & Shipping	Store product at 2 - 4°C for up to 6 months

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Assay Protocol

Reaction Components

Active Protease (Catalog #: T575-31AN)

Immobilized Trypsin, Agarose.

Digestion Buffer (User Prepared)

50 mM Tris-HCl pH 8

Reaction Protocol

The following conditions may be different for different proteins. Optimize the protocol for each specific protein. Protein of interest must be purified prior to digestion and dialyzed with digestion buffer.

- Step 1.** Prepare protein sample in digestion buffer at the concentration range of 1-10 mg/ml
- Step 2.** Gently resuspend resin and aliquot 15 μ l of the uniformed resin to a microfuge tube
- Step 3.** Spin tube at 500 x g and remove supernatant
- Step 4.** Add 500 μ l of digestion buffer and resuspend
- Step 5.** Spin tube at 500 x g and remove supernatant
- Step 6.** Repeat Steps 4 and 5 twice
- Step 7.** Resuspend the resin with 200 μ l of digestion buffer
- Step 8.** Add 800 μ l of protein sample to the resin and mix well
- Step 9.** Incubate reactions in a platform shaker at room temperature for 1-16 hours
- Step 10.** Monitor the cleavage products by SDS-PAGE or HPLC
- Step 11.** Elute protein via one of two methods when desired reaction is achieved
 - a. Centrifugation at 500 x g for 3 minutes and collect supernatant; or
 - b. Transfer the contents of the tube to a column and collect eluate
- Step 12.** In both methods above, a second elution can be performed with 200 μ l of digestion buffer
- Step 13.** To regenerate resin:
 - a. Wash the resin with 1 ml of deionized water
 - b. Repeat step a twice
 - c. Resuspend the resin in 50% glycerol with 50 mM Tris-HCl, pH 8
 - d. Store at 2-4°C.

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