

Product Information

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Aminopeptidase I from *Streptomyces griseus*

Catalog Number **A9934**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

CAS RN 9031-94-1

EC 3.4.11.22

Synonym: Leucine aminopeptidase IV

Product Description

Aminopeptidase I from *S. griseus* has a fairly broad specificity, being able to remove the N-terminal residue of all proteins, except where the penultimate residue is an imino acid. As such, it may be used as a reagent for analysis of protein structure. It may also serve as a model for studies of proteolytic enzyme activation by calcium ions.

This heat-stable aminopeptidase with an N-terminal Ala-Pro-Asp-Ile-Pro-Leu sequence is a monomeric zinc metalloprotein. The approximate molecular mass of Aminopeptidase I has been determined:⁵ 21 kDa (gel filtration) and 33 kDa (SDS-PAGE). The enzyme contains two Zn^{2+} binding sites and is inhibited by 1,10-phenanthroline. It is activated six-fold by Ca^{2+} , which also stabilizes it against heat inactivation. The isoelectric point (pI) is 5.4.

Aminopeptidase I may also be used as a reagent in the assay of endoprotease activities with a synthetic substrate in a two-stage assay. In the first stage, the endoprotease cleaves a peptide, such as Z-Y-X-Leu-*p*-nitroanilide, with the X, Y, and Z residues being chosen according to the specificity of the endoprotease. After termination of the first stage (by heating, changing pH, or adding a specific inhibitor), the aminopeptidase is added, and the Leu-*p*-nitroanilide, product of the endoprotease reaction, is quantified by determining the amount of *p*-nitroaniline generated by measuring the absorption at 405 nm. As an alternative to the two-stage reaction, the aminopeptidase may be present, in excess, with the endoprotease, and the rate of increase in absorbance at 405 nm will be a measure of the action of the endoprotease.

The product is supplied as a lyophilized powder with a protein content of 40–60% (Lowry). The lyophilized powder also contains calcium acetate.

Specific Activity: ≥ 200 units/mg protein.

Unit Definition: One unit will hydrolyze 1.0 μmole of L-leucine-*p*-nitroanilide to L-leucine and *p*-nitroaniline per minute at pH 8.0 at $25\text{ }^{\circ}\text{C}$ and 3.0 mM substrate concentration.

Endopeptidase content: ≤ 0.01 unit/mg protein
(as μmole tyrosine equivalent per minute released from casein.)

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

Reconstitute in 20 mM tricine, pH 8.0, with 0.05% bovine serum albumin. Dilute the enzyme with the reconstitution buffer to 0.15–0.30 unit/ml for a working concentration. Solutions should be prepared fresh prior to use.

Storage/Stability

Store the product desiccated at $-20\text{ }^{\circ}\text{C}$. Under these conditions the product retains activity for at least two years.

References

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5. Spungin, A., and Blumberg, S., *Eur. J. Biochem.*, **183**, 471-477 (1989).
6. Greenblatt, H.M. et al., *J. Mol. Biol.*, **265**, 620-636 (1997).

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