

Pfu Methionine Aminopeptidase

Code No. 7335

Size : 20 mU (at 37°C)

Shipping at -20

Stored at -20

Lot No. K201-1
Concentration : 0.20 mU/μl (at 37°C)
Activities : 1.5 U/mg protein at 37°C
13.3 U/mg protein at 75°C
Expiry Date : June 2003

Source : *Escherichia coli* carrying plasmids encoding the *Pyrococcus furiosus* methionine aminopeptidase gene.

Description : Pfu Methionine Aminopeptidase is a thermostable Methionine Aminopeptidase, which specifically liberates the N-terminal methionine residue from proteins and peptides.

Purity : More than 95% pure as determined by SDS-PAGE.
No other proteases detected.

Form : Solution in 10 mM Tris-HCl, pH7.5, containing 0.01 % Tween 20, 0.1 mM CoCl₂

Definition of Activity : One unit of enzyme activity corresponds to the amount required to produce 1 μmol of Met from Met-Pro-Ala-Ala-Gly in 1 minute at pH 7.5.

Storage : -20 °C

Properties :

- Molecular weight : 32,848 (Mass spectrum analysis)
37,000 (SDS-PAGE)
- Optimum temperature : 85-95 °C
- Thermal stability : This enzyme is stable at 75 °C
(pH7.2, 1 hour, 0.5 mM Co²⁺).
- Optimum pH : 7.0 - 8.0
- Stable pH range : This enzyme is stable at pH 4.1 - 10.6
(75 °C, 1 hour, 0.5 mM Co²⁺).
- Tolerance to denaturants :
Stable against ≤ 2 M urea
≤ 0.2 M guanidine- HCl
≤ 0.01% SDS
- Activator : Co²⁺
- Inhibitor : EDTA

Substrate specificity

X	Cleavage rate (100 % with Met - Ala - Ala - Ala)
Ala	100
Gly	58
Ser	18
Thr	9.1
Pro	34
Val	2.9

The cleavage rate of Methionine was examined by using synthetic peptides Met-X-Ala-Ala-Ala(X;20 amino acids)as substrate. Pfu Methionine Aminopeptidase does not react on other amino acids than the above (Cys, Asp, Asn,Leu, Ile, Gln, Glu, His, Met, Phe, Lys, Tyr, Trp, Arg).

Application example : Liberation of N-terminal Methionine residue from recombinant human fibronectin fragment C281
(MW: 33,000, Met - Ala - Pro- Thr- Asp - Leu · · ·)

Reaction condition : Substrate : 680 pmol
Enzyme amount : 2 mU
Reaction buffer : 10 mM PIPES buffer, pH 7.5,
containing 0.2 mM CoCl₂
Reaction volume : 200 μl

Amino acid sequence was analyzed by a sequencer after incubation at 75 °C for 5 hours .

Note : The Protein sample should be denatured by chemical procedure such as carboxymethylation.

References :

- 1) Ben-Bassat, A., Bauer, K., *et al.*(1987) *J. Bacteriol.*, **169**, 751.
- 2) Miller, C.G., Strauch, K.L., *et al.*(1987) *Proc. Natl.Acad.Sci.* **84**, 2718.
- 3) Ben-Bassat, A., Bauer, K., *et al.*(1987) *Nature*, **326**, 315.
- 4) Yasueda, H., Nagase, K. *et al.*(1990) *Bio/Technology*, **8**, 1036.
- 5) Tunasawa, S., Izu, Y., Miyagi, M. and Kato, I. (1997) *J. Biochemistry*, **122**, 843-850.

Note : For research use only. Not for use in diagnostic or therapeutic procedures.

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