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Product Information

PROTEASE, BACTERIAL, TYPE VIII Sigma Prod. No. P 5380

CAS NUMBER: 9014-01-1

ENZYME COMMISSION NO.: 3.4.21.62

SYNONYMS: Subtilisin A, Subtilisin Carlsberg, Subtilopeptidase A

STRUCTURE:

It is a single polypeptide chain of MW 27,287 (complete amino acid sequence available on request).²

PHYSICAL DESCRIPTION:

Isoelectric point: pI = 9.4

$E^{1\%}(280\text{nm}) = 8.6$

Sedimentation coefficient: $S_{20,w} = 2.77^3$

STABILITY / STORAGE AS SUPPLIED:

This protease is offered as a white powder, essentially free of buffer salts. It is quite stable stored frozen. Supplier studies indicate that the solid will lose less than 10% of its activity per year if stored at 4°C. Long-term storage in excess of 20°C is to be avoided.

SOLUBILITY / SOLUTION STABILITY:

The product is readily soluble in water at all concentrations which occur in normal usage, to give a colorless solution.² Sigma tests it in 10 mM sodium acetate buffer, containing 5 mM calcium acetate, pH 7.5, for assay purposes, and obtains a clear solution.¹

Subtilisin A is reportedly active in some organic solvents, dry octane, for example.⁴

This enzyme is stable 1-2 days at 4°C as a 100-200 mg/mL solution in 0.1 M borate, pH 8.0, containing 0.1 M calcium chloride.³

UNIT DEFINITION:

One unit will hydrolyze casein to produce color equivalent to 1.0 μmole (181 μg) of tyrosine per minute at pH 7.5 at 37°C (color by Folin-Ciocalteu reagent).

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Sigma Prod. No. P 5380

Activity: 7-15 units per mg solid. Each lot is checked to justify the catalog statement "substantially free of DNase and RNase."¹

The activity of this enzyme is sometimes given in Anson units. (One Anson unit is defined as the amount of enzyme which, under specified conditions, digests urea-denatured hemoglobin at an initial rate such that there is liberated an amount of TCA-soluble product per minute which gives the same color with Folin-Ciocalteu Phenol reagent as one milliequivalent of tyrosine. For this enzyme, the "specified conditions" were 25°C, pH 7.50 and reaction time of 10 minutes.)² Based on average values, one Anson unit = 370 Sigma units.¹

GENERAL DESCRIPTION:

This is a proteolytic enzyme isolated from the fermentation of *Bacillus licheniformis*. It is a serine endoproteinase with a broad specificity towards native and denatured proteins, and is active under alkaline conditions.

OPTIMAL CONDITIONS:²

Effect of pH at constant temperature (T=25°C), for 10 minutes (activity remaining):

pH 6 ≈ 70%	pH 10.5 ≈ 90%
pH 7 ≈ 80%	pH 11 ≈ 70%
pH 7.5-10 ≈ 95%	pH 11.5 ≈ 0%

Effect of temperature at constant pH (pH=8.5), for 10 minutes (activity remaining):

30°C ≈ 25%	55-60°C ≈ 95+%
40°C ≈ 40%	65°C ≈ 80-85%
50°C ≈ 75%	70°C ≈ 15%

Effect of temperature at constant pH (pH=8.5), for 1 hour (activity remaining or relative stability):

at 50°C, > 95% activity remaining after 60 minutes
at 55°C, ≈ 90% after 60 minutes
at 60°C, ≈ 80% after 60 minutes
at 65°C, ≈ 75% after 10 minutes, ≈ 50% after 20 minutes, ≈ 20% after 60 minutes
at 70°C, ≈ 50% after 5 minutes, ≈ 25% after 10 minutes ≈ 0% after 35 minutes

Effect of pH at constant temperature (T=25°C) for 24 hours. (activity remaining or relative stability):

at pH 5, ≈ 20%	at pH 8-10, ≈ 90%
at pH 6, ≈ 50%	at pH 11, ≈ 45%
at pH 7, ≈ 75%	at pH 11.5, ≈ 0%

PROTEASE, BACTERIAL, TYPE VIII
Sigma Prod. No. P 5380

REFERENCES:

1. Sigma quality control.
2. Supplier information.
3. P. Boyer, *The Enzymes*, ed., vol. III, 3rd ed., p. 564.
4. Zaks, A. and Klibanov, A.M., *J. Biol. Chem.*, 263, 3194-3201 (1988).

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Crystal structure of subtilisin Carlsberg.

Jacobs, Myra, et al., *Nucleic Acids Research*, 13, 8913-8926 (1985). Cloning, sequencing and expression of subtilisin Carlsberg.

Ottesen, M. and Svendsen, I., *Methods in Enzymology*, XIX, 199. Subtilisins.

Hannappel, E., et al., *Archives of Biochem. Biophysics*, 214, 293-298 (1982). Limited proteolysis of aldolases.