

Product Information

Protease from *Bacillus licheniformis*

Type VIII, lyophilized powder

Catalog Number **P5380**

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

CAS RN 9014-01-1

EC 3.4.21.62

Synonyms: Subtilisin A, Subtilisin Carlsberg, Subtilopeptidase A, Proteinase from *Bacillus licheniformis*

Product Description

This proteolytic enzyme is isolated from *Bacillus licheniformis*.¹ Known by various names, such as Subtilisin A and Subtilisin Carlsberg, this protease is a serine endoproteinase with a broad specificity towards native and denatured proteins, and is active under alkaline conditions.² Subtilisin A is a single polypeptide chain of molecular mass of ~ 27 kDa. Several publications have elucidated the sequence of this enzyme.³⁻⁷ The crystal structure of native Subtilisin Carlsberg has been reported.⁸

Studies on the use of Subtilisin A in non-aqueous, organic solvents have been published.⁹⁻¹⁰

Isoelectric point (pI):¹¹ 9.4

E1% (280nm) :¹¹ 8.6

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 $37\text{ }^{\circ}\text{C}$ (color by Folin-Ciocalteu reagent).

Preparation Instructions

The product is generally soluble in water at normal usage concentrations. Different publications report preparation of stock solutions of this product at various concentrations:

- 1.1 mg/mL in 10 mM Trizma buffer (pH 8.0)¹²
- 100 mg/mL in DPBS (without added calcium or magnesium)¹³

Storage/Stability

This enzyme is reported to be stable for 1-2 days at $4\text{ }^{\circ}\text{C}$ as a 100-200 mg/mL solution in 0.1 M borate (pH 8.0), containing 0.1 M CaCl_2 .¹¹ Stock solutions of this product may be frozen at $-20\text{ }^{\circ}\text{C}$.¹⁴

Optimal Conditions¹⁵

Effect of pH at constant temperature ($T = 25\text{ }^{\circ}\text{C}$), for 10 minutes (activity remaining):

- pH 6 $\approx 70\%$
- pH 7 $\approx 80\%$
- pH 7.5-10 $\approx 95\%$
- pH 10.5 $\approx 90\%$
- pH 11 $\approx 70\%$
- pH 11.5 $\approx 0\%$

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

- $30\text{ }^{\circ}\text{C} \approx 25\%$
- $40\text{ }^{\circ}\text{C} \approx 40\%$
- $50\text{ }^{\circ}\text{C} \approx 75\%$
- $55-60\text{ }^{\circ}\text{C} \approx 95+\%$
- $65\text{ }^{\circ}\text{C} \approx 80-85\%$
- $70\text{ }^{\circ}\text{C} \approx 15\%$

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

- At $50\text{ }^{\circ}\text{C}$, $> 95\%$ activity remaining after 60 min.
- At $55\text{ }^{\circ}\text{C}$, $\approx 90\%$ after 60 min.
- At $60\text{ }^{\circ}\text{C}$, $\approx 80\%$ after 60 min.
- At $65\text{ }^{\circ}\text{C}$, $\approx 75\%$ after 10 min., $\approx 50\%$ after 20 min., $\approx 20\%$ after 60 min.
- At $70\text{ }^{\circ}\text{C}$, $\approx 50\%$ after 5 min., $\approx 25\%$ after 10 min., $\approx 0\%$ after 35 min.

Effect of pH at constant temperature ($T = 25\text{ }^{\circ}\text{C}$) for 24 hours. (activity remaining or relative stability):

- pH 5, $\approx 20\%$
- pH 6, $\approx 50\%$
- pH 7, $\approx 75\%$
- pH 8-10, $\approx 90\%$
- pH 11, $\approx 45\%$
- pH 11.5, $\approx 0\%$

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

References

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- (15) Supplier data (2020).

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