Product Information

Protease from *Bacillus licheniformis*

Type VIII, lyophilized powder

P5380

Product Description

CAS Registry Number: 9014-01-1

Enzyme Commission (EC) Number: 3.4.21.62

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

Isoelectric point (pI):[1] 9.4

$E^{1\%}$ (280 nm):[11] 8.6

This proteolytic enzyme is isolated from *Bacillus licheniformis*.[2] 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. It is active under alkaline conditions.[3] Subtilisin A is a single polypeptide chain of molecular mass of ~ 27 kDa. Several publications have elucidated the sequence of this enzyme.[4-8] The crystal structure of native Subtilisin Carlsberg has been reported.[9]

Studies on the use of Subtilisin A in non-aqueous, organic solvents have been published.[10-11] Several theses[12] and dissertations[13-15] have cited use of product P5380 in their research protocols.

Reagent

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).

Storage/Stability

This product should be stored in its lyophilized form at –20 °C.

This enzyme is reported to be stable for 1-2 days at 4 °C as a 100-200 mg/mL solution in 0.1 M borate (pH 8.0) buffer which contains 0.1 M $CaCl_2$.[1] Stock solutions of this product may be frozen at –20 °C.[16]

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.

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 Tris buffer (pH 8.0)[17]
- 100 mg/mL in DPBS (without added calcium or magnesium)[18]

Optimal Conditions

Effect of pH at constant temperature (T = 25 °C), for 10 minutes (activity remaining):[19]

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

Effect of temperature at constant pH (pH = 8.5), for 10 minutes (activity remaining):[19]

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

Effect of temperature at constant pH (pH = 8.5), for 1 hour (activity remaining or relative stability):[19]

- 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):[19]

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

References

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