TECHNICAL DATASHEET

SUBTILISIN-DFE PURE100

Name of the enzyme: **SUBTILISIN-DFE**
Organism of origin: *Bacillus amyloliquefaciens*
Recombinant production in: *Escherichia coli* Rosetta (DE3) pLysS (Novagen; Ref: 70956), the enzyme contains a 6-histidine tag in its C-terminus end.

CAS: 9014-01-1
ENZYME COMMISSION NUMBER: 3.4.21.62
SYNONYMS: Sub.DFE-His

PHYSICAL DESCRIPTION:
Appearance: white powder
Form: Lyophilized powder
Quality: Nickel Affinity Chromatography
Storage Temperature: -20ºC/-80ºC

SPECIFICITY: Subtilisin has a broad specificity for peptide bond cleavage, with preference for a large uncharged residue at P1 and low activity for glutamyl residues; it hydrolyses peptide amides. The enzyme has active-site geometry similar to the trypsin family but otherwise there is no sequence homology. Subtilisins belong to subtilases, a group of serine proteases that initiate the nucleophilic attack on the peptide (amide) bond through a serine residue at the active site[1].

PROPERTIES:

Molecular weight	**45-27,5**
Optimum pH:	**7,5-8,0**
Isoelectric point:	**6,65**
Optimum Tª (ºC)	**37**

COMPOSITION:

Identifiers	Number	Name	Concentration in the lyophized
CAS number	9014-01-1	Sub.DFE-His	6000 Units
CAS number	7647-14-5	NaCl	100 mM
E.	339	Na_2HPO_4 and NaH_2PO_4	20 mM
CAS number	60-24-2	β-Mercaptoethanol	1 mM

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SUBTILISIN-DFE PURE100

PURE100 SUBTILISIN-DFE is purified by two step Nickel Affinity Chromatography and Hiprep desalting, highly pure (>95%). For this reason, ***PURE100 SUBTILISIN-DFE*** is suitable for applications in research and biomedicine.

ACTIVATORS:

Subtilisin is activated by Ca^{+2} and Mn^{+2}. Calcium salts improve the thermostability of the enzyme.

INHIBITORS:

Subtilisin is partially inhibited by some amino acids (nitrogen source) which inhibit the production of alkaline proteases[34].
Furthermore, di-isopropyl fluorophosphates, a protease inhibitor prepared from potato markedly inactivates the enzyme. Slightly inactivated by surface-active agents such as sodium dodecylsulfate[5].

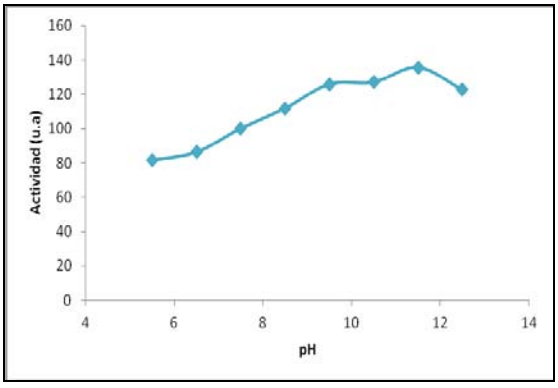
SUBSTRATES:

PURE100 SUBTILISIN-DFE is soluble in water or aqueous buffers.

PURE100 SUBTILISIN-DFE is a serine endoproteinase with a broad specificity towards native and denatured proteins; used in transesterification and transpeptidation reactions. It acts at random inner-peptides of proteins to produce smaller peptides of these molecules rapidly.

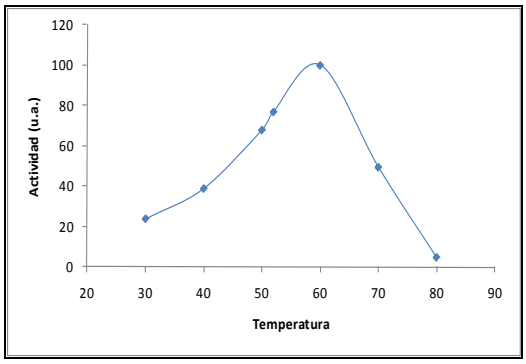
ENZYMATIC ACTIVITY AND CHARACTERIZATION

PURE100 SUBTILISIN-DFE activity is > 1000 U/mg with Tris-HCl buffer (using casein as substrate and at 37 ºC) depending on the pH range.



The activity of PURE100 SUBTILISIN-DFE is relativized to percent of activity at different pHs

The best enzymatic activity is observed at 60ºC where the enzyme shows good stability.



The activity of PURE100 SUBTILISIN-DFEis relativized to percent of activity at different temperatures

APPLICATIONS:

PURE100 SUBTILISIN-DFE - Subtilisins are widely used in commercial products[6], such as:

- Detergents: laundry, dishwashing, cleaning of membrane systems, clean surgical instruments
- Cosmetics
- Food processing
- Skin care ointments
- Contact lens cleaners
- Leather industry
- And for research purposes in synthetic organic chemistry.

METHODS OF PREPARATION:

PURE100 SUBTILISIN-DFE is provided as a lyophilized powder and remains stable without any significant loss of activity when stored at -20ºC/-80ºC. We recommend dissolving the enzyme immediately before using it or to store in aliquots at -20ºC for better preservation of the activity. We recommend avoiding multiple freeze-thaw cycles and exposure to frequent temperature changes.

PURE100 SUBTILISIN-DFE is provided in two formats, 1.500 U and 6.000 U. The enzyme is soluble in water and diluted salts solution; depending of the application of the enzyme can be dissolved in both.

The re-constitutive buffer of the enzyme is composed by 20 mM phosphate buffer pH 7.5.
We recommend to dissolve the enzyme in 1 ml of re-constitutive buffer in order to make an enzymatic stock solution (1.500 U/ml and 6.000 U/ml respectively) and aliquot for storing at -20ºC/-80ºC.

Stock solution must be diluted in the re-constitutive buffer or can be directly added into the solution where the enzyme is going to be working, in order to achieve the required enzymatic activity.
The working solution must be prepared just prior usage and remains stable if stored at 2-8ºC for 2-3 days or for long-term use, if frozen at -20ºC for better preservation of the original activity.

STABILITY/STORAGE AS SUPPLIED

This product is stable for at least one year when stored at -80°C.

SOLUTION/SOLUTION STABILITY

Usually, solutions are prepared in phosphate buffer (20 Mm phosphate buffer, pH 7.5). If the application permits we recommend adding 1 mM β-Mercaptoethanol at the working solution for avoiding possible enzymatic precipitation.

UNIT DEFINITIONS:

“One unit of enzyme is able to hydrolyze casein resulting in an absorbance value as the Folin-Ciocalteu reagent equivalent to 1 µmol (181 µg) of tyrosine per minute at pH = 7.5 at 37 º C"

REFERENCES

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[5] R. Gupta, Q.K. Beg, P. Lorenz. Bacterial alkaline proteases: molecular approaches and industrial applications. Appl Microbiol Biotechnol. 59 (1):15-32, 2002
[6] R. Gupta, Q.K. Beg, P. Lorenz. Bacterial alkaline proteases: molecular approaches and industrial applications. Appl Microbiol Biotechnol. 59 (1):15-32, 2002

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