

Pronase

Nuclease-free, isolated from *Streptomyces griseus*
Lyophilized powder

Cat. No. 10 165 921 001 1 g

Cat. No. 11 459 643 001 5 g

 **Version 08**

Content version: May 2019

Store at +2 to +8°C

Product description

Introduction

Pronase is a non-specific protease. Its proteolytic activity extends to both denatured and native proteins, which in general are broken down into individual amino acids (1). The composition typically contains neutral protease, chymotrypsin, trypsin, carboxypeptidase, and aminopeptidase, together with neutral and alkaline phosphatases. The preparation is free from nucleases. The molecular weights of individual components lie between 20,000 and 60,000 D.

Application

- Histochemistry and cell culture (breaking down tissue)
- Glycobiology (analysis of glycoproteins) (2)
- Molecular genetics (extraction of phage DNA, isolation of plasmid DNA) (3)

Advantages of the product

Nuclease-free, according to the current Quality Control procedures.

Composition

The preparation contains 20% calcium acetate for stability. It is free of starch according to the current Quality Control procedures.

Units

The unit of non-specific protease activity is in general the enzyme activity that increases the rate of release of folin-positive amino acids and peptides from casein at a temperature of 40°C and pH 7.5 by a quantity equivalent to a given amount of tyrosine per minute. For the unit designated 'U', that quantity of tyrosine is 1 µmol/min, for the unit 'PU' it is 1 µg/min (1 U 181 PU); for the unit 'PUK' it is 0.1/min (change of absorbance of molybdenum blue, formed by reaction with folin's reagent, under conditions such that 1 PUK = 50 PU).

Specific activity of pronase

The specific activity of the lyophilized powder is 7.0 U/mg, equivalent to approx. 1270 PU/mg or approx. 25 PUK/mg.

Specificity

Pronase has a broad specificity, breaking down virtually all proteins into their individual amino acids. Table 1 gives the yields for various proteins, taking the amount of primary amine nitrogen released by digestion with 6 M hydrochloric acid for 24 h at +110°C as equivalent to the amount present in the protein. Enzymatic hydrolysis was carried out for 72 h at +40°C and pH 7.4.

Table 1

Protein	Nitrogen from primary amine released (mg/g)		Yield (in %)
	Enzymatic hydrolysis	Acid hydrolysis	
Casein	69.6	93.0	74.8
Ovalbumin	78.6	89.4	87.9
Gluten	62.5	78.4	79.7

Stability

The lyophilized powder is stable stored dry at +2 to +8°C until the expiration date printed on the label.

Solubility

The lyophilisate is soluble in H₂O (10 – 20 mg/ml).

Stock solution

Stock solution is prepared by adding pronase powder to water (10 – 20 mg/ml). An aqueous solution (10 mg/ml) is stable for at least 2 h at +55°C; it is assumed that it can be kept for about two weeks at +2 to +8 °C and a year at –15 to –25°C.

Working concentration

Prepare a solution containing 0.1 M Tris (pH 7.5) and 0.5% SDS, heat it to +35 to +40°C and add enough stock pronase solution (10 – 20 mg/ml) to give a solution in which the concentration of pronase is 0.5 – 2.0 mg/ml. For prolonged digestion, the solution should contain 10 mM CaCl₂.

Enzyme properties

Molecular weight

The molecular weights of the various enzymes present are 20,000 – 60,000 Da.

pH Optimum

The optimum value is 7.0 – 8.0, but the preparation is very stable in the pH range 6.0 – 9.0. Outside the range 4.0 – 10.0, pronase is unstable.

Temperature optimum

The temperature optimum is in the range +40 to +60°C. Even in dilute solution, pronase is not very temperature-sensitive: it loses its activity at +80°C, but 10 min at +60°C reduces its activity by only 30% in casein solutions.

Inhibitors

Since pronase contains a variety of proteases and other enzymes, it is advisable to employ a mixture of different types of protease inhibitor, such as the following:

Table 2

Inhibitor	Inhibitor specificity	Working concentration
Leupeptin*	Serine and thiol proteases	0.5 mg/l
EDTA-Na ₂	Metalloprotease	1 mM
Pepstatin*	Acid proteases	0.7 µg/ml
PMSF*	Serine proteases	0.2 mM
Pefabloc ¹⁾ SC	Serine proteases	0.8 mM
Protease Inhibitor Set	Broad spectrum	
cOmplete	Broad spectrum	1 tablet/50 ml
cOmplete mini	Broad spectrum	1 tablet/10 ml

Pronase is inhibited by Cu²⁺ and inactivated by acids, soaps and hydrogenperoxide.

References

- 1 Narahashi, Y. (1970) *Methods Enzymol.* **19**, 651.
- 2 Spiro, R.G. (1966) *Methods Enzymol.* **8**, 26.
- 3 Maniatis, T., Fritsch, E.F. & Sambrook, J. (1989) *Molecular Cloning – A Laboratory Manual*, Kapitel 2.8.0, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- 4 Trop, M. & Birk, Y. (1970) *Biochem. J.* **116**, 19–25.

Changes to previous version

Editorial changes.

Ordering Information

Product	Cat. No.	Pack size
Proteinase K, lyophilizate	03115801001	2 × 250 mg
	03115836001	25 mg
	03115852001	4 × 250 mg
	03115879001	100 mg
Proteinase K, solution	03115828001	5 ml
	03115844001	25 ml
	03115887001	1.25 ml
Leupeptin	11 017 101 001	5 mg
	11 017 128 001	25 mg
	11 034 626 001	50 mg
	11 529 048 001	100 mg
Pefabloc SC	11 429 868 001	100 mg
	11 585 916 001	500 mg
	11 429 876 001	1 g
Pepstatin	10 253 286 001	2 mg
	11 359 053 001	10 mg
	11 524 488 001	50 mg
PMSF	10 236 608 001	1 g
	10 837 091 001	10 g
	11 359 061 001	25 g
Protease Inhibitors Set	11 206 893 001	Set consisting of 3 mg Antipain-2HCl, 0.5 mg Bestatin, 1 mg Chymostatin, 3 mg E-64, 5 mg Leupeptin, 0.5 mg Pepstatin, 3 mg Phosphoramidon, 20 mg Pefabloc SC, 10 mg EDTA, 0.5 mg Aprotinin
cOmplete Protease Inhibitor Cocktail Tablets	11 697 498 001	20 tablets
	11 836 145 001	3 x 20 tablets
cOmplete, EDTA-free	11 873 580 001	20 tablets
cOmplete mini	11 836 153 001	25 tablets
cOmplete mini, EDTA-free	11 836 170 001	25 tablets
Universal Protease Substrate (casein, resorufin-labeled)	11 080 733 001	15 mg
	11 734 334 001	40 mg

* available from Roche Diagnostics

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