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# **Pronase** from *Streptomyces griseus*

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Lyophilized powder

**Cat. No. 10 165 921 001** 1 g **Cat. No. 11 459 643 001** 5 g

Store the product at +2 to +8°C.

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#### 1. General Information

#### 1.1. Contents

Streptomyces griseus • The composition typically contains neutral protease, chymotrypsin, trypsin, carboxypeptidase, 11 459 643 00	Vial / Bottle	Label	Function / Description	Catalog Number	Content
and aminopeptidase, together with neutral and alkaline phosphatases. Contains 20% calcium acetate for stability.  i The preparation is free of starch according to the current Quality Control procedures.	1	Streptomyces	<ul> <li>The composition typically contains neutral protease, chymotrypsin, trypsin, carboxypeptidase, and aminopeptidase, together with neutral and alkaline phosphatases. Contains 20% calcium acetate for stability.</li> <li>The preparation is free of starch according to the</li> </ul>	10 165 921 001 11 459 643 001	1 vial, 1 g 1 vial, 5 g

## 1.2. Storage and Stability

#### **Storage Conditions (Product)**

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Pronase	Store at +2 to +8°C.  • Store dry.

#### **Storage Conditions (Working Solution)**

An aqueous solution (10 mg/ml) is stable for at least 2 hours at  $+55^{\circ}$ C; it is assumed that it can be kept for about two weeks at +2 to  $+8^{\circ}$ C and a year at -15 to  $-25^{\circ}$ C.

#### Reconstitution

Stock solution is prepared by adding Pronase powder to double-distilled water (10 to 20 mg/ml).

## 1.3. Additional Equipment and Reagent required

#### For preparation of working concentration

- Sodium dodecyl sulfate (SDS)\*
- Tris-HCI\*

#### For protease inhibition

- SDS\*
- Leupeptin\*
- Pepstatin\*
- PMSF
- Pefabloc SC\*
- Protease Inhibitor Set\*
- cOmplete protease inhibitors\*

## 1.4. Application

Use Pronase for

- Histochemistry and cell culture to break down the tissue.
- Glycobiology (analysis of glycoproteins).
- Molecular genetics (extraction of phage DNA, isolation of plasmid DNA).

#### 2. How to Use this Product

#### 2.1. Parameters

#### **Contaminants**

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

#### Inhibition

Since Pronase contains a variety of proteases and other enzymes, it is advisable to add a mixture of different types of protease inhibitors:

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

<sup>?</sup> Pronase is inhibited by Cu<sup>2</sup>+ and inactivated by acids, soaps, and hydrogen peroxide.

### **Molecular Weight**

The molecular weight of the various enzymes present is 20,000 to 60,000 Da.

## pH Optimum

pH 7.0 to 8.0

## pH Stability

The preparation is stable in the pH range 6.0 to 9.0. Outside the range of 4.0 to 10.0, Pronase is unstable.

## **Specific Activity**

The specific activity of the lyophilized powder is 7 U/mg, equivalent to approximately 1,270 PU/mg or approximately 25 PUK/mg.

## **Specificity**

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

Protein	Nitrogen from primary amine released [mg/g]		Value [%]
	Enzymatic hydrolysis	Acid hydrolysis	
Casein	69.9	93.0	74.8
Ovalbumin	78.6	89.4	87.9
Gluten	62.5	78.4	79.7

#### **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 minutes at +60°C reduces its activity by only 30% in casein solutions.

#### **Unit Definition**

The unit of nonspecific 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 (U), that quantity of tyrosine is 1 µmol/minute.
- For the unit (PU), it is 1 µg/minute (1 U 181 PU).
- For the unit (PUK), it is 0.1/minute (change of absorbance of molybdenum blue, formed by reaction with folin's reagent, under conditions such that 1 PUK = 50 PU).

#### Working Concentration

- 1 Prepare a solution containing 0.1 M Tris\*, pH 7.5 and 0.5% SDS\*.
- 2 Heat to +35 to +40°C and add enough stock Pronase solution (10 to 20 mg/ml) to give a solution in which the concentration of Pronase is 0.5 to 2.0 mg/ml.

⚠ For prolonged digestion, add 10 mM CaCl, to the solution.

## 3. Additional Information on this Product

#### 3.1. Test Principle

Pronase is a nonspecific protease. Its proteolytic activity extends to both denatured and native proteins, which in general are broken down into individual amino acid.

# 4. Supplementary Information

#### 4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols			
1 Information Note: Additional information about the current topic or procedure.			
⚠ Important Note: Information critical to the success of the current procedure or use of the product.			
1 2 3 etc.	Stages in a process that usually occur in the order listed.		
1 2 3 etc.	Steps in a procedure that must be performed in the order listed.		
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.		

# 4.2. Changes to previous version

Layout changes. Editorial changes.

# 4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
Tris hydrochloride	500 g	10 812 846 001
Leupeptin	5 mg	11 017 101 001
	25 mg	11 017 128 001
	50 mg	11 034 626 001
	100 mg	11 529 048 001
Pepstatin	2 mg	10 253 286 001
	10 mg	11 359 053 001
	50 mg	11 524 488 001
PMSF	10 g	10 837 091 001
	25 g	11 359 061 001
Pefabloc® SC	100 mg	11 429 868 001
	500 mg	11 585 916 001
	1 g	11 429 876 001
Protease Inhibitors Set	1 set, 10 individual protease inhibitors	11 206 893 001
cOmplete	20 tablets in a glass vial, for 50 ml each	11 697 498 001
	3 x 20 tablets in glass vials, for 50 ml each	11 836 145 001
cOmplete, Mini	25 tablets in a glass vial, for 10 ml each	11 836 153 001

#### 4.4. Trademarks

All product names and trademarks are the property of their respective owners.

#### 4.5. License Disclaimer

For patent license limitations for individual products please refer to: **List of biochemical reagent products**.

## 4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

## 4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

# 4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.