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Not for use in diagnostic procedures.



# Aprotinin from bovine lung

 **Version: 10**

Content Version: October 2019

Lyophilized

<b>Cat. No. 10 236 624 001</b>	10 mg
<b>Cat. No. 10 981 532 001</b>	50 mg
<b>Cat. No. 11 583 794 001</b>	100 mg

**Store the lyophilizate at +2 to +8°C.**

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# 1. General Information

## 1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Aprotinin	Trypsin inhibitor, pancreas type (BPTI)	10 236 624 001	1 vial, 10 mg
			10 981 532 001	1 vial, 50 mg
			11 583 794 001	1 vial, 100 mg

## 1.2. Storage and Stability

### Storage Conditions (Product)

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

Vial / Bottle	Label	Storage
1	Aprotinin	Store at +2 to +8°C. <b>⚠ Store dry.</b>

### Storage Conditions (Working Solution)

Store an aqueous solution of Aprotinin adjusted to pH 7 to 8 for approximately 1 week at +2 to +8°C. Alternatively, the solution can be stored in aliquots for ≥6 months at –15 to –25°C.

**⚠ Avoid repeated freezing and thawing and exposure to strongly alkaline solutions (inactive at pH >12.8)**

### Reconstitution

Aprotinin is soluble in water (10 mg/ml) and aqueous buffer solutions, such as 0.1 M Tris buffer, pH 8.0.

## 1.3. Application

Aprotinin can be used in the following applications:

- Protection of proteins and enzymes during isolation and purification.
- Purification of, for example, urokinase, trypsin, and chymotrypsin on immobilized Aprotinin.
- Quantification of kallikrein activity in mixtures of esterases and proteases.
- Guarantee the controlled degradation of substrates by avoiding nonspecific proteolysis in clinical chemical tests.
- Inhibition of protease activity and thus, the increase of the lifetime of cells in cell and tissue-culture studies.
- As a model protein in protein folding studies.
- Size determination (molecular weight marker) in SDS-polyacrylamide gel electrophoresis.

## 2. How to Use this Product

### 2.1. Before you Begin


#### General Considerations

##### Classes of proteases

Proteases can be assigned to various classes on the basis of their characteristic active centers:

Protease Type	Active Center
Serine	Serine and histidine
Cysteine	Cysteine (thiol, SH-)
Metallo	Metal ions, such as Zn <sup>2+</sup> , Ca <sup>2+</sup> , Mn <sup>2+</sup>
Aspartate	Aspartic acid moiety

##### Protease classes and their specific inhibitors

Serine	Cysteine	Metallo	Aspartate
Aprotinin*	E-64*	Bestatin (aminopeptidase)*	Pepstatin*
Pefabloc® SC*		Phosphoramidon	
Pefabloc® SC PLUS*			
Leupeptin*			
 <i>Inhibits serine and cysteine proteases with trypsin-like specificity.</i>			
PMSF*			
cOmplete Protease Inhibitor Cocktail Tablets, EDTA-free*			
cOmplete Protease Inhibitor Cocktail tablets*			
α2-Macroglobulin* (endoproteinases)			

## 2.2. Parameters

### Absorbance

$$A_{280 \text{ nm}, 1 \text{ cm}} (1 \text{ mg/ml}) = 0.84$$

### Inhibition

#### Mechanism of inhibition

Aprotinin is a competitive inhibitor that forms a loose complex with serine proteases and blocks their active centers. The complex exhibits many interactions between protease and inhibitor. The trypsin-aprotinin complex, for example, does not dissociate at a concentration of 8 M uric acid or 6 M guanidine hydrochloride. Aprotinin is only cleaved slowly by most proteases, with the exception of thermolysin at temperatures  $> +60^{\circ}\text{C}$ .

### Isoelectric Point

10.5

### Molecular Weight

6,512 Da

### pH Stability

Inactive at pH  $> 12.8$ .

### Purity

Electrophoretically homogeneous. Impurities are not detectable by SDS-PAGE.

### Sequence

#### Structure

- Aprotinin is a monomeric polypeptide of 58 amino acids with the following sequence:  
RPDFCLEPPY TGPKARIIR YFYNAKAGLC  
QTFVYGGCRA KRNNFKSAEN CMRTCGGA
- The three-dimensional conformation is maintained by three disulfidebridges Cys<sub>5</sub>-Cys<sub>55</sub>, Cys<sub>14</sub>-Cys<sub>38</sub>, and Cys<sub>30</sub>-Cys<sub>51</sub>.
- In the trypsin-aprotinin complex, the following amino acids appear to be responsible for the strong binding of the inhibitor to the enzyme: lysine at position 15 and alanine at position 16 in the active center of the enzyme and two arginine moieties at positions 17 and 39.

## 2. How to Use this Product

### Specificity

Aprotinin inhibits serine proteases. It inhibits kallikrein, the protease that releases hypotensive peptides, such as

- Kallidin and bradykinin (human plasma kallikrein:  $K_i = 3 \times 10^{-8}$  M at pH 8.0, porcine pancreas kallikrein:  $K_i = 1 \times 10^{-9}$  M at pH 8.0)
- Trypsin ( $K_i = 2.8 \times 10^{-11}$  M at pH 7.8,  $K_i = 2.6 \times 10^{-9}$  M at pH 4.0, non-competitive)
- Trypsinogen, chymotrypsin ( $K_i = 9 \times 10^{-9}$  M at pH 8.0)
- Bacterial fibrinolysin and plasmin ( $K_i = 1$  nM at pH 7.3)

Aprotinin weakly inhibits:

- Cathepsin G
- Acrosin
- Human leukocyte elastase
- Human urokinase

Aprotinin shows no inhibition of:

- Factor Xa
- Thrombin
- Subtilisin
- Papain
- Pepsin
- Angiotensin-converting enzyme (ACE)
- Carboxypeptidase A and B
- Other metalloproteases
- Thiolproteases

See section, **General Considerations** for a table of protease classes and their inhibitors.

### Unit Definition

One inhibitor unit (IU) is defined as the amount of Aprotinin that completely inhibits 1 U trypsin in <10 minutes at pH 6. Trypsin activity determined at +25°C, pH 8.0, BAEE as substrate. One inhibitor unit (IU) corresponds to approximately:

- 2.8 inhibitor units (+25°C, Chromozym TRY as substrate).
- 26 kallikrein inhibitor units (KIU) (+25°C).
- 0.067 inhibitor units (+25°C; Bz-D, L-Arg-4-Na as substrate, trypsin determination at pH 7.8).

One kallikrein inhibitor unit = 0.17 µg crystalline Aprotinin.

### Working Concentration

0.06 to 2 µg/ml (0.01 to 0.3 µM)

**⚠ To avoid adsorption of Aprotinin onto negatively charged solid phases, such as chromatography gels or ultra-filtration membranes, use a NaCl concentration >0.1 M or add other suitable salts to all buffers used during the separation.**

## 3. Additional Information on this Product

### 3.1. Test Principle

Aprotinin, also known as pancreatic trypsin inhibitor and trypsin-kallikrein inhibitor, is found in a number of organs:

- Lungs
- Spleen
- Liver
- Pancreas

It is also detectable in a free form in calf serum. An intracellular form has also been identified. Its unique structure is responsible for the molecule's high stability and remarkable resistance to elevated temperatures, acids, and proteases.

#### Reversibility

Aprotinin binding to serine proteases is weak and dissociates in alkaline (pH 10) and acid environments (pH 5 for most proteases, pH <3 for trypsin and plasmin). At these extreme pH values, it should therefore be possible to separate Aprotinin from the protease by chromatography on a molecular-sieve column or by filter dialysis.



### 3.2. Quality Control

Function tested with an excess of trypsin.

## 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	
	<i>Information Note: Additional information about the current topic or procedure.</i>
	<b>Important Note: Information critical to the success of the current procedure or use of the product.</b>
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.
Non-finished products		
cOplete, EDTA free	7500 tablets in glass vial	04 574 834 001
Reagents, kits		
E-64, Protease Inhibitor	10 mg	10 874 523 001
	25 mg	11 585 681 001
Pefabloc® SC (AEBSF)	custom fill	11 427 393 103
cOplete	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
	20 tablets, for 50 ml each	04 693 116 001
cOplete, Mini	25 tablets in a glass vial, for 10 ml each	11 836 153 001
	30 tablets, for 10 ml each	04 693 124 001
cOplete, Mini, EDTA-free	25 tablets in a glass vial, for 10 ml each	11 836 170 001
Leupeptin	custom fill	10 528 595 103
cOplete, Mini, EDTA-free	30 tablets, for 10 ml each	04 693 159 001
Pepstatin	custom fill	10 253 294 103
cOplete, EDTA-free	20 tablets, for 50 ml each	04 693 132 001
Bestatin	10 mg	10 874 515 001
Pefabloc® SC PLUS	Set I, 100 mg Pefabloc® SC; 5 ml PSC-Protector solution	11 873 601 001
	Set II, 1 g Pefabloc® SC; 2 x 25 ml PSC-Protector solution	11 873 628 001
α <sub>2</sub> -Macroglobulin	25 inhibitor units	10 602 442 001
PMSF	10 g	10 837 091 001
	25 g	11 359 061 001



## 4.4. Trademarks

COMPLETE is a trademark of Roche.

All other 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.

