



The Celltechgen™ Mammalian Protein Extraction Reagent

(Store at 2°C-8°C)

CTG-PA0015-A	The Celltechgen™ Aprotin,25mg	ISSUE DATE 9 July 2019
CTG-PA0015-B	The Celltechgen™ Aprotin,100mg	ISSUE DATE 9 July 2019

Contents:Lyophilizate

Product description: Trypsin inhibitor, pancreas type

Structure: Aprotinin is a monomeric polypeptide (58 amino acids) with the following sequence:

RPDFCLEPPY TGPCKARIIR YFYNAKAGLC

QTFVYGGCRA KRNNFKSAEN CMRTCGGA

The two-dimensional conformation is maintained by three disulfidebridges Cys₅-Cys₅₅, Cys₁₄-Cys₃₈ und Cys₃₀-Cys₅₁.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. (7,8,9).

Properties

Molecular weight	Mr = 6512
Isoelectric point	10.5
Absorbance Solubility	A280 nm, 1 cm (1 mg/ml) = 0.84
Solubility	Freely soluble in water (10mg/ml) and aqueous buffer solution(e.g.0.1 M Tris buffer, pH 8.0).

Typical analysis	Function-tested with an excess of trypsin
Purity	Electrophoretically homogeneous
Impurities	Not detectable by SDS-PAGE
Recommended	0.06-2.0ug/ml(0.01-0.3uM)
Working concentration	Note:To avoid adsorption of aprotinin onto negatively charged solid phases (e.g.chomatography gels,ultra-filtration membranes)the Nacl concentration should be above 0.1M or other suitable salts should be added to all buffers used during the separation.
Storage/Stability	The lyophilizate is stable when stored dry +2°C to+8°Cuntil the expiration date printed on the lable.An aqueous solution of aprotinin (adjusted to PH7-8) is stable for about 1 week at +2to+8°C。 The solution can be stored in aliquots for at least 6 months at -15 °C to-25 °C.Avoid repeated freezing and thawing and exposure to strongly alkaline solution(inactive at PH>12.8)

Description Aprotin,also known as pancreatic trypsin inhibitor and trypsin-kallikrein inhitor,is found in a number of organs,e.g.

- Lungs
- spleen
- Liver and
- pancreas

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

Classes of proteases Proteases can be assigned to various classes on the basis of their characteristic active centers.

- Serine proteases with serine and histidine in the active center.
- Cysteine proteases with cysteine (thiol, SH-) in the active center.
- Metalloproteases with metal ions (e.g. Zn²⁺, Ca²⁺, Mn²⁺) in the active center.
- Aspartate proteases with an aspartic acid moiety in the active center

- Application**
- Protection of proteins and enzymes during isolation/purification,
 - Purification, e.g. of urokinase, trypsin and chymo-trypsin on immobilized aprotinin,
 - Quantification of kallikrein activity in mixtures of esterases and proteases,
 - To guarantee the controlled degradation of sub- strates by avoiding unspecific proteolysis in clinical chemical tests,
 - Inhibition of protease activity and thus increase of the lifetime of cells in cell and tissue culture studies,
 - Aprotinin as a model protein in protein folding studies,
 - Molecular weight marker in SDS-polyacrylamide gel electrophoresis.

Table 1 shows these protease classes and their specific inhibitors

Serine proteases	Cysteine proteases	Metalloproteases	Aspartate proteases
APMSF		Bestatin (amino peptidase)	Pepstatin
AntithrombinIII			
α_1 -Antitrypsin(α_1 -protease inhibitor)	E-64	EDTA-Na ₂	
Aprotinin Isocoumarin		Phosphoramidon	
Pefabloc® SC			
Leupeptin(inhibits serine and cysteine proteases with trypsinlike specificity)			
PMSF			
cOmplete Protease Inhibitor Cocktail tablets			
α_2 .Macroglobulin(endoproteinases)			

Table1:Protease classes and their inhibitors

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 (exception: thermolysin at temperatures above +60 °C)

Reversibility	Aprotinin binding to serine proteases is weak and dissociates in alkaline (pH 10) and acid environments (pH5 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.
Unite definition	One inhibitor unit(IU) is desined as the amount of aprotinin that completely inhibits 1U trypsin in <10min at PH6.(Trypsin activity determined at 25 °C , PH8,BAEE as substrate) One inhibitor unit(IU)(+25 °C , BAEE as substrate)corresponds to about 2.8inhibitor units(+25 °C , Chromozym TRY as substrate) One inhibitor units(IU)(+25 °C , BAEE as substrate)corresponds to about 26 kallikrein inhibitor units(KIU)(+25 °C) One inhibitor unit(IU)(+25 °C , BAEE as substrate) corresponds to about 0.067 inhibitor units(+25 °C ; Bz-D,L-Arg-4-Na as substrate,trypsin determination at PH7.8) .One kallikrein inhibitor unit=0.17ug crystalline
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