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TECHNICAL INFORMATION

Catalog Number: 190382, 190779, 191158, 194101, 194559
Aprotinin

Molecular Formula: $C_{284}H_{432}N_{84}O_{79}S_7$

Molecular Weight: Approximately 6511.23

CAS # 9087-70-1

Synonyms: Antagosan; Anikrein; Antilysin(e); Basic pancreatic trypsin inhibitor; BPTI; Bayer A 128; Kallikrein-trypsin inactivator; Fosten; Iniprol; Kunitz protease inhibitor; Onquinin; Repulson; RP-9921; Ryker 52G; Trasylol; Triazinin; Zymofren

Physical Description: White lyopilized powder

pI: 10.5[10]

$E^{1\%}$ (280 nm) = 8.3 (water)

Source: Bovine Lung

Unit Definitions:

KIU: One KIU (Kallikrein Inactivating Unit) is identical to the quantity of protease inhibitor that has the ability to inhibit 2 Kallikrein units by 50% under optimal conditions.

TIU: One trypsin inhibitor unit (TIU) is the amount of inhibitor per mg which hydrolyzes one umole of benozyl-DL-arginine-p-nitroanilide (BAPNA) per minute. One TIU reduces the activity of two trypsin units by 50%. **Note:** When benzoyl-L-arginine ethyl ester (BAEE) is used as the substrate, one BAPNA unit is approximately 45 BAEE umolar units at pH 8.0 at 25°C or approximately 9000 BAEE A_{253} units at pH 7.6 at 25°C.

One Inhibitor Unit - 1500 KIU

One TIU = 900 KIU

One TIU = 8800 Schwert and Takenake units (STU)

Solubility: Soluble in water (10 mg/ml - completely soluble) and in aqueous buffers of low ionic strengths.[8] Dilute solutions are generally less stable than concentrated ones. Solution stability also depends on pH; values of 1-12 can be tolerated.[18] Repeated freeze-thaw cycles should be avoided. Sterilization of solutions can be done by filtration through a 0.2 um filter.

Description: Found in bovine lymph nodes, lung, parotid gland, spleen, liver, pancreas, seminal vesicles, thyroid gland, kidney, mucous membranes of the trachea and esophagus, ovaries, heart, posterior pituitary and cartilage.[9,17,18]

A serine protease inhibitor which is reactive against trypsin, chymotrypsin, plasmatic and glandular kininogenases, plasmin, kallkrein, urokinase, clotting factor XIIa, protein C, proteinases of the complement system, and leukocyte and tissue proteinases. It does not inhibit thrombin. Aprotinin works by blocking the active sites of enzymes. Binding is reversible with most aprotinin-protease complexes dissociating at pH > 10 or < 3.

Enzyme - Source - Condition	**Inhibition (K_i = Dissociation Constant)**
Acrosin	Weak Inhibition[8]

Chymotrpsin	K_i = 9 nM[16]
Chymotrypsinogen - bovine - pH 8.0	K_i = 9 nM[18]
CMP-N-Acetylneuraminate lactosylceramide a-2,3-sialytransferase	74% Inhibition at 300 nM[16]
Elastase - human leukocytes - pH 8.0	K_i = 3.5 uM[18]
Kallikrein - pancreatic - pH 8.0	K_i = 1.0 nM[18]
Kallikrein - plasma	K_i = 30 nM; 100 nM[16]
Kallikrein - tissue	K_i = 1 nM[16]
Kallikrein - urine	K_i = 1.7 nM[16]
Plasmin - porcine - pH 7.8	K_i = 4.0 nM[18]
Plasminogen activator	K_i = 8 uM; 27 uM[16]
Trypsin - bovine - pH 8.0	K_i = 0.06 pM[18]
Trpsinogen - bovine - pH 8.0	K_i = 1.8 uM[18]
Tryptase TL-2	16% Inhibition at 10 uM[16]
Urokinase - human - pH 8.8	K_i = 8.0 uM[18]

Effective Concentration: Equimolar with protease (1-2 ug/ml).

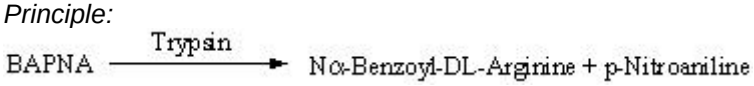
Stability: Aprotinin is relatively stable to high temperature, acids, alkali, organic solvents and proteolytic digestion (only thermolysin has been found capable of degrading aprotinin after heating to 60-80°C).[18] The Cyc[14]-Cys[38] disulfide bridge is readily split by reducing agents like b-mercaptoethanol.[18]

Solvent	**Concentration**	**Storage Temperature**	**% Loss/Time**
Saline solution with 0.9% benzyl alcohol, pH 5.7 to 6.2	10 mg/ml	+4°C	< 4.3% per year
2.5% Trichloroacetic acid	N/A	80°C	No Loss[9]
pH < 12.6	N/A	N/A	No loss observed after 24 hours[19]
pH > 12	N/A	N/A	Irreversibly denatured[20]

pH 7-8	0.065-1.95 ug/ml	+4°C	About 1 week[8]
pH 7-8	0.065-1.95 ug/ml	-20°C	> 6 months[8]

TIU Assay Procedure:

Principle:



BAPNA = Na-Benzoyl-DL-Arginine-p-nitroanilide

Conditions: T = 25°C, pH = 7.8, A_{405nm}, Light path = 1 cm

Method: Continuous spectrophotometric determination.

Reagents:

A. 200 mM Triethanolamine buffer with 20 mM calcium chloride, pH 7.8 at 25°C: Prepare 100 ml in deionized water using triethanolamine hydrochloride and calcium chloride, dihydrate. Adjust to pH 7.8 at 25°C with 1 M NaOH.
B. 0.1% (w/v) Na-Benzoyl-DL-arginine-p-arginine-p-nitroanilide solution (BAPNA): Prepare 25 ml in deionized water using Na -Benzoyl-DL-arginine-p-nitroanilide, hydrochloride. Heat solution to not greater than 65°C to facilitate solubilization. Prepare solutions fresh each time. Solutions must be completely solubilized before use. **Note:** If the solution is hazy, continue to stir over gentle heat until the solution becomes clear. Do not use the solution if it turns yellow - this indicates possilbe chemical decomposition of the substrate due to overheating.
C. 1 mM Hydrochloric acid solution: Prepare 50 ml in deionized water.
D. Trypsin Enzyme Solution: Prepare by dissolving 2.5 mg Trypsin in 20 ml of cold reagent C. Prepare fresh each time.
E. 0.9% (w/v) sodium chloride solution (NaCl): Prepare 100 ml in deionized water.
F. Aprotinin Inhibitor Solution: Prepare three separate aprotinin solutions in Reagent E, each containing 0.047 to 0.06 trypsin inhibitor units per ml. Use a separate solution for each replication of assay. **Note:** The % inhibition must be between 40 and 60 percent for the assay to be valid. Adjust the concentration of the inhibitor solution so that the results are obtained in this range.

Procedure:

Pipette the following reagents into suitable cuvettes:

	Uninhibited Test	**Inhibited Test**	**Blank**
Reagent A	1.60 ml	1.60 ml	1.60 ml
Reagent C	--	--	0.20 ml
Reagent D	0.20 ml	0.20 ml	--
Reagent E	0.20 ml	--	0.20 ml
Reagent F	--	0.20 ml	--

Mix by inversion and equilibrate to 25°C. Monitor the A_{405nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent B	1.00 ml	1.00 ml	1.00 ml

Immediately mix by inversion and record the increase in A_{405nm} for approximately 5 minutes. Obtain the DA_{405nm}/minute using

the maximum linear rate for the Uninhibited, Inhibited, and Blank Solutions.

Calculations:

$$\text{TIU/ml} = \frac{(\Delta A_{405nm}\text{/minute Uninhibited} - \Delta A_{405nm}\text{/minute Inhibited})(\text{df})}{(9.96)(\text{ml Aprotinin/ml RM})}$$

TIU = Trypsin Inhibitor Units
df = Dilution factor
9.96 = The millimolar extinction coefficient of p-Nitroaniline at 405 nm
RM = Reaction Mix

$$\text{TIU/mg solid} = \frac{\text{TIU/ml}}{\text{mg solid/ml}}$$

$$\text{\% Inhibition} = \frac{\Delta A_{405nm}\text{/minute Uninhibited} - \Delta A_{405nm}\text{/minute Inhibited} \times 100}{\Delta A_{405nm}\text{/minute Uninhibited} - \Delta A_{405nm}\text{/minute Blank}}$$

Note: In cases where there is variability in the results, ensure that the uninhibited rate has a DA of 0.08 to 0.12. This may be required to reduce the variance caused by the range in specific activity of the Trypsin used.

Final Assay Concentration:

In a 3.00 ml reaction mix, the final concentrations are 107 mM triethanolamine, 11 mM calcium chloride, 0.03% (w/v) BAPNA, 0.07 mM hydrochloric acid, 0.025 mg trypsin, 0.12% (w/v) sodium chloride, 0.0003% (w/v) thimerosal and 0.0094-0.012 trypsin inhibitor unit of aprotinin.

Availability:

Catalog Number	Description	Size
190382	Aprotinin, lyophilized, Activity approximately 6000 KIU per mg	10 mg 50 mg
190779	Aprotinin, Aseptically filled solution in 0.9% NaCl and 0.9% benzyl alcohol; Activity 100,000 KIU/10 ml ampule	10 ml 4 × 10 ml
191158	Aprotinin, lyophilized; Activity approximately 3-4 Inhibitor units/mg dry weight	1 mg 5 mg 10 mg 25 mg 100 mg 250 mg
194101	Aprotinin, lyophilized; Activity not less than 3 TIU/mg	5 mg 25 mg 100 mg
194559	Aprotinin, Cell Culture Reagent, lyophilized; Activity approximately 3-4 Inhibitor units/mg of dry weight	10 mg 25 mg 100 mg 250 mg

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