

## Product Information

### Protease Inhibitor Panel

Product Code **INHIB1**  
 Storage Temperature  $-20\text{ }^{\circ}\text{C}$

## TECHNICAL BULLETIN

### Product Description

Create your own broad-spectrum protease inhibitor cocktails or screen your extracts for proteolytic activity. The INHIB-1 panel includes inhibitors for serine, cysteine, and acid proteases, calpains, and metalloproteinases.

Panel components also include economical alternatives, such as NEM, EACA, EDTA, and Soybean Trypsin Inhibitor.

### Reagents

Protease Inhibitor	Product Code	Package Size	Storage Temp	Working Range	Molecular Weight	Stock Solution Solubility
AEBSF	A8456	25 mg	$-20\text{ }^{\circ}\text{C}$	0.1–1 mM	239.7	50 mg/mL (water)
6-Aminohexanoic acid	A2504	25 g	RT	5 mg/mL	131.2	25 mg/mL (water)
Antipain	A6191	5 mg	$-20\text{ }^{\circ}\text{C}$	1–100 $\mu\text{M}$	604.7	50 mg/mL (water)
Aprotinin	A1153	5 mg	2–8 $^{\circ}\text{C}$	10–800 nM	6512	10 mg/mL (water)
Benzamidine HCl	B6506	5 g	2–8 $^{\circ}\text{C}$	0.5–4 mM	156.6	50 mg/mL (water)
Bestatin	B8385	5 mg	$-20\text{ }^{\circ}\text{C}$	40 $\mu\text{M}$	344.8	25 mg/mL (water)
Chymostatin	C7268	5 mg	$-20\text{ }^{\circ}\text{C}$	10–200 $\mu\text{g/mL}$ (10–100 $\mu\text{M}$ )	Approx 608	6 mg/mL (DMSO)
E-64	E3132	5 mg	2–8 $^{\circ}\text{C}$	10 $\mu\text{M}$	357.4	20 mg/mL (water)
EDTA disodium salt	ED2SS	50 g	RT	1 mM	372.2	50 mg/mL (water)
N-Ethylmaleimide	E3876	5 g	2–8 $^{\circ}\text{C}$	0.1–1 mM	125.1	50 mg/mL (ethanol)
Leupeptin	L2884	5 mg	$-20\text{ }^{\circ}\text{C}$	10–100 $\mu\text{M}$	475.6	50 mg/mL (water)
Pepstatin	P5318	5 mg	2–8 $^{\circ}\text{C}$	0.5–1.0 $\mu\text{g/mL}$	685.9	1 mg/mL (ethanol)
Phosphoramidon	R7385	5 mg	$-20\text{ }^{\circ}\text{C}$	10 $\mu\text{M}$	543.5 (free acid)	10 mg/mL (water)
Trypsin inhibitor	T9003	100 mg	$-20\text{ }^{\circ}\text{C}$	1:1 stoichiometric binding	20,100	10 mg/mL (water)

### Preparation Instructions

Stock solutions of each inhibitor should be prepared first, prior to creating a cocktail. Mixtures of some inhibitor stock solutions may result in precipitation due to interactions between inhibitors and the mixing of solvents. In most cases further dilution will aid solubility.

### Storage/Stability

As powders all reagents can be stored at  $-20\text{ }^{\circ}\text{C}$ . Those reagents designated for room temperature and 2–8  $^{\circ}\text{C}$  storage do not require storage at  $-20\text{ }^{\circ}\text{C}$ , but will not be adversely affected when stored at  $-20\text{ }^{\circ}\text{C}$ . Allow all powders to warm to room temperature before opening. Store tightly sealed and protect from moisture.

#### **4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride**

Product Number **A8456**

Acronym: AEBSF

##### **Product Description**

AEBSF is a serine protease inhibitor.<sup>1,3,4,5</sup> Inhibition constants for AEBSF are similar to those of PMSF and DFP.<sup>1</sup> AEBSF has been shown to inhibit trypsin,<sup>1</sup> chymotrypsin,<sup>1</sup> plasmin,<sup>1</sup> kallikrein,<sup>1,5</sup> and thrombin.<sup>1,3,4</sup> As an alternative to PMSF and DIFP, AEBSF offers lower toxicity, improved solubility in water, and improved solution stability particularly in aqueous systems.<sup>1</sup> The LD<sub>50</sub> determined from oral doses in mice for AEBSF is higher than those for DFP and PMSF.<sup>1</sup> AEBSF has been used in cell culture at concentrations up to 0.25 mM.<sup>1</sup> Our recommended working concentration range is 0.1 mM to 1 mM.

##### **Preparation Instructions**

AEBSF is directly soluble in water. Solutions in water are slightly acidic and retain inhibitory activity for up to six months when stored refrigerated. Solutions at pH above 7 are less stable.<sup>1,2</sup> Stock solutions should be stored at a pH less than 7. If a final pH of greater than 7 is required, the pH should be adjusted shortly before use.

#### **6-Aminohexanoic acid**

Product Number **A2504**

Synonyms:  $\epsilon$ -Aminocaproic Acid; EACA

##### **Product Description**

EACA is reported to inhibit chymotrypsin, Factor VIIa, lysine carboxypeptidase, plasmin, and plasminogen activator.<sup>6</sup>

##### **Preparation Instructions**

EACA is directly soluble in water at 25 mg/mL. As an inhibitor of plasmin<sup>7</sup> it has been utilized in the clotting buffer for fibrinogen assays. This buffer is 10 mM potassium and sodium phosphate, pH 6.4, with 0.20 g CaCl<sub>2</sub>, 5 g 6-Aminohexanoic acid, 1 g sodium azide, and 9 g NaCl in 1 liter. The buffer is stable indefinitely at room temperature.

#### **Antipain hydrochloride**

Product Number **A6191**

Synonyms: [(S)-1-carboxy-2-phenylethyl]-carbamoyl-L-arginyl-L-valyl-arginal; N-(N $\alpha$ -carboxyl-Arg-Val-Arg-al)-Phe

##### **Product Description**

Isolated from a microbial source, antipain hydrochloride is a reversible inhibitor of serine/cysteine proteases and some trypsin-like serine proteases.<sup>8,9,10</sup> Its action resembles leupeptin; however, its plasmin inhibition is less and its cathepsin A inhibition is more than that observed with leupeptin. Concentrations for 50% inhibition ( $\mu$ g/mL): papain, 0.16; trypsin, 0.26; cathepsin A, 1.19; cathepsin B, 0.59; cathepsin D, 125; plasmin, >93; chymotrypsin and pepsin, >250<sup>8,9</sup>. It also has been reported to inhibit calpain I, (porcine) with K<sub>i</sub> = 1.4  $\mu$ M.<sup>11</sup>

##### **Preparation Instructions**

Solubility testing at 50 mg/mL in water yields a clear to slightly hazy yellow solution. It is reportedly soluble in methanol, water, and DMSO; less soluble in ethanol, butanol, and propanol; insoluble in benzene, hexane, and chloroform.<sup>8</sup> A stock solution in water or buffer is stable for at least a week at 4 °C and for about a month at -20 °C.<sup>9</sup>

Dilute solutions should be stored on ice and kept for only a day because of the terminal aldehyde, which is subject to oxidation and racemization.

## **Aprotinin**

Product Number **A1153**

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

### **Product Description**

Aprotinin is a competitive serine protease inhibitor which forms stable complexes with and blocks the active sites of enzymes. The binding is reversible and most aprotinin-protease complexes dissociate at pH >10 or <3.<sup>12</sup>

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

Unit Definition: One Trypsin Inhibitor Unit (TIU) will decrease the activity of 2 trypsin units by 50%, where 1 trypsin unit will hydrolyze 1.0  $\mu\text{mole}$  of N $\alpha$ -benzoyl-DL-arginine p-nitroanilide (BAPNA) per minute at pH 7.8 and 25 °C. Another commonly used unit of activity is the KIU (Kallikrein Inhibitor Unit). A conversion factor for these Aprotinin units is: 1 TIU = 1,300 KIU.<sup>14</sup> Another conversion factor is: 1 TIU = 1,025 KIU.<sup>17</sup>

### **Preparation Instructions**

Aprotinin is freely soluble in water (>10 mg/mL) and in aqueous buffers of low ionic strengths.<sup>14,15</sup> Dilute solutions are generally less stable than concentrated ones. Solution stability is pH dependent; a range of 1-12 can be tolerated.<sup>13</sup> Repeated freeze-thaw cycles should be avoided. Due to its compact tertiary structure, aprotinin is relatively stable against denaturation due to high temperature, pH extremes, organic solvents, or proteolytic degradation (only thermolysin has been found capable of degrading aprotinin after heating to 60–80 °C).<sup>13</sup> The high basicity of aprotinin causes it to adhere to commonly used dialysis tubing and even gel filtration matrices, but the use of acetylated materials and concentrated salt solutions ( $\geq 0.1 \text{ M NaCl}$  in buffer) minimizes the problem.<sup>13</sup> Sterilization may be achieved by filtration through a 0.2  $\mu\text{m}$  filter.<sup>14</sup>

<b>Enzyme</b>	<b>Inhibition</b>
Acrosin	Weak inhibition <sup>15</sup>
Chymotrypsin	$K_i = 9 \text{ nM}^{16}$
Chymotrypsinogen (bovine), pH 8.0	$K_i = 9 \text{ nM}^{13}$
CMP-Sialic Acid: Lactosylceramide $\alpha$ -(2,3)-Sialyltransferase	74% Inhibition at 300 nM <sup>16</sup>
Elastase (human leukocytes), pH 8.0	$K_i = 3.5 \mu\text{M}^{13}$
Kallikrein (pancreatic), pH 8.0	$K_i = 1.0 \text{ nM}^{13}$
Kallikrein (plasma)	$K_i = 30 \text{ nM}; 100 \text{ nM}^{16}$
Kallikrein (tissue)	$K_i = 1 \text{ nM}^{16}$
Kallikrein (urine)	$K_i = 1.7 \text{ nM}^{16}$
Plasmin (porcine), pH 7.8	$K_i = 4.0 \text{ nM}^{13}$
Plasminogen activator	$K_i = 8 \mu\text{M}; 27 \mu\text{M}^{16}$
Trypsin (bovine), pH 8.0	$K_i = 0.06 \mu\text{M}^{13}$
Trypsinogen (bovine), pH 8.0	$K_i = 1.8 \mu\text{M}^{13}$
Tryptase TL-2	16% Inhibition at 10 $\mu\text{M}^{16}$
Urokinase (human), pH 8.8	$K_i = 8.0 \mu\text{M}^{13}$

## **Benzamidine hydrochloride hydrate**

Product Number **B6506**

### **Product Description**

Benzamidine HCl is a reversible inhibitor of trypsin, trypsin-like enzymes, and serine proteases.<sup>18,19,20</sup> A concentration of approximately 1 mM is used for general protease inhibition. To inhibit proteases from yeast, a range of 0.5 to 4.0 mM is used and it is for the most part interchangeable with pepstatin A.<sup>21</sup>

In addition to being a strong competitive inhibitor of trypsin, benzamidine HCl has been also shown to be a strong competitive inhibitor of thrombin and plasmin. It was also found to be as effective as aprotinin in the prevention of glucagon degradation in human plasma.<sup>22</sup>

### **Preparation Instructions**

Benzamidine HCl is soluble in water and alcohol.<sup>20</sup> Solubility testing in water at a concentration of 50 mg/mL yields a clear solution with heating. Benzamidine HCl is sensitive to oxidation. It is recommended to prepare solutions fresh each time in degassed water prior to use. However, frozen aliquots stored under inert gas, to exclude air, may be stable for a short time. Insufficient information is available to assess the shelf-life of a frozen solution.

## **Bestatin hydrochloride**

Product Number **B8385**

### **Product Description**

Bestatin is a competitive and specific inhibitor of leucine aminopeptidase, aminopeptidase B, and triamino peptidase. It inhibits aminopeptidase B at 60 nM (using arginine- $\beta$ -naphthylamide as substrate) and leucine aminopeptidase at 20 nM (leucine- $\beta$ -naphthylamide as substrate).<sup>23</sup> It showed no inhibition of aminopeptidase A, trypsin, chymotrypsin, elastase, papain, pepsin, or themolysin.<sup>24</sup>

### **Preparation Instructions**

Solubility testing in water at 25 mg/mL yields a clear solution. Stock solutions at 1 mM are expected to be stable at least 1 month stored at  $-20\text{ }^{\circ}\text{C}$ .

## **Chymostatin**

Product Number **C7268**

### **Product Description**

Chymostatin is a mixture of several components, typically 79–89% chymostatin A, 12–17% chymostatin B and 5–15% chymostatin C.<sup>25</sup>

Chymostatin A MW = 607.7

Chymostatin B MW = 593.7

Chymostatin C MW = 607.7

Chymostatin is a strong inhibitor of many proteases, including chymotrypsin, chymotrypsin-like serine proteinases, chymases, and lysosomal cysteine proteinases such as cathepsins B, H and L.<sup>28,29</sup> It weakly inhibits human leukocyte elastase.<sup>30</sup> It is effective at a final concentration of 100–200  $\mu\text{g/mL}$  (10–100  $\mu\text{M}$ ). Chymostatin is often included in protease inhibitor cocktails used with plant extracts.<sup>27</sup>

### **Preparation Instructions**

Solubility testing in glacial acetic acid at 10 mg/mL yields a clear solution, which is usually colorless, but can be yellow in appearance.<sup>14</sup>

It is reportedly also soluble in DMSO; only slightly soluble in water and short-chain alcohols; insoluble in ethyl acetate, butyl acetate, ether, hexane, and petroleum ether.<sup>26,27</sup> Stock solutions (10 mM) can be prepared in DMSO and are stable for months at  $-20\text{ }^{\circ}\text{C}$ . Stock solutions can also be made in 0.1 M HCl. Dilute solutions (10–100  $\mu\text{M}$ ) are only stable for several hours, due to oxidation of the terminal aldehyde.<sup>28</sup>

**trans-Epoxy succinyl-L-leucylamido-(4-guanidino)butane**

Product Number **E3132**

Synonyms: E-64; Proteinase Inhibitor E 64; N-[N-(L-3-transcarboxyirane-2-carbonyl)-L-Leucyl]-agmatine<sup>1</sup>

**Product Description**

E-64 is an irreversible, potent, and highly selective cysteine protease inhibitor. E-64 does not react with the functional thiol group of non-protease enzymes, such as L-lactate dehydrogenase or creatine kinase.<sup>32,35</sup> E-64 will not inhibit serine proteases (except trypsin) like other cysteine protease inhibitors, leupeptin and antipain.<sup>38,40</sup> The trans-epoxy succinyl group (active moiety) of E-64 irreversibly binds to an active thiol group in many cysteine proteases, such as papain, actinidase, and cathepsins B, H, and L to form a thioether linkage.<sup>40,46</sup> E-64 is a very useful cysteine protease inhibitor for use in *in vivo* studies because it has a specific inhibition, it is permeable in cells and tissues, it has low toxicity, and it is stable.<sup>40</sup>

E-64 inhibits the following enzymes at the indicated concentrations:

Actinidin<sup>36</sup>  
Ananain<sup>37</sup> (pineapple stem);  
Bromelain (stem, 10  $\mu$ M and fruit)<sup>32,38</sup>  
Calpain (chicken skeletal muscle)<sup>39</sup>  
Cathepsin B (human and rat liver, 10  $\mu$ M)<sup>32,35,38,40-42</sup>  
Cathepsin B1 (squid, 10  $\mu$ M)<sup>43</sup>  
Cathepsin H (human liver, 10  $\mu$ M)<sup>38,40,41</sup>  
Cathepsin L (human, 10  $\mu$ M, and rat liver)<sup>35,38,40-42,44</sup>  
Cathepsin (rat liver, 2.8 mM, about 82% inhibition)<sup>45</sup>  
Clostripain (100  $\mu$ M, 81% reversible competitive inhibition)<sup>38,46</sup>  
Comosain (pineapple stem)<sup>37</sup>  
CMP-Sialic Acid:Lactosylceramide  
 $\alpha$ (2-3)-Sialyltransferase (SAT-1)<sup>47</sup>  
Ficin (10  $\mu$ M)<sup>38</sup>  
a-Ginivain<sup>46</sup>  
Papain (10  $\mu$ M)<sup>32,38,48</sup> (E-64 was not overcome by high levels of cysteine, by dialysis or by gel filtration<sup>32</sup>)  
 $\alpha$ - and  $\beta$ -Trypsin (the latter by a reversible competitive mechanism)

E-64 is reported to be one of the most effective low molecular weight inhibitors of trypsin catalyzed hydrolysis.<sup>46</sup> E-64 inhibited the activity of bleomycin hydrolase and blocked the activity of a yeast cysteine protease gene (YCP1), which induces an increase in bleomycin metabolism (this may be the cause of

bleomycin resistance during bleomycin therapeutic treatment).<sup>49</sup> E-64 (100  $\mu$ g/mL) promoted heat-induced apoptosis in mouse mammary carcinoma FM3A cells.<sup>33</sup> E-64 ( $\geq 10$   $\mu$ M) inhibited neutrophil movement (chemotaxis) induced by C5a, suggesting that an active thiol protease is needed for chemotaxis to C5a.<sup>50</sup> E-64 (50–100  $\mu$ M) selectively blocked T cell receptor-triggered programmed cell death in a mouse hybridoma.<sup>34</sup> E-64 inhibited the ability of EJ human bladder carcinoma cells to invade through an artificial basement membrane (probably by inhibition of cathepsin B) and to degrade the human basement membrane laminin.<sup>51</sup>

**Preparation Instructions**

E-64 is soluble in water. A 20 mg/mL solution can be prepared in deionized water (heat may be needed).<sup>14</sup> A suggested stock solution is a 1 mM aqueous solution. The effective concentration for use as a protease inhibitor is 1–10  $\mu$ M.<sup>31</sup> Aqueous stock solutions are stable for months at  $-20$   $^{\circ}$ C. Diluted solutions are stable for days at neutral pH.<sup>31</sup> E-64 is stable from pH 2–10, but is unstable in ammonia or in HCl.<sup>32</sup> E-64 is also soluble in DMSO,<sup>33</sup> a 10 mM solution can be prepared in dry DMSO and stored at  $-20$   $^{\circ}$ C. Subsequent dilutions were in culture medium.<sup>34</sup> Solutions for injection were prepared by dissolving E-64 in 0.9% sodium chloride or in a minimum amount of saturated sodium bicarbonate followed by dilution with 0.9% sodium chloride (after adjusting the pH to 7.0 with acetic acid).<sup>35</sup>

**Ethylenediaminetetraacetic acid disodium dihydrate**

Product Code **ED2SS**

Synonym: EDTA

**Product Description**

Zinc-dependent metalloproteinases, as well as other proteases that are stabilized by calcium, can be effectively inhibited by chelation of divalent metal ions with EDTA. Other chelators such as EGTA, specific for calcium, and 1,10-phenanthroline, highly specific for zinc, can be used to target these two different types of proteases.

**Preparation Instructions**

The disodium salt of EDTA is soluble in water at room temperature up to 0.26 M. The pH of this solution will be approximately 4–6.

## **N-Ethylmaleimide**

Product Number **E3876**

Synonyms: NEM

### **Product Description**

NEM is an inhibitor of cysteine proteases such as calpain.<sup>54</sup> NEM binds stoichiometrically and is also used as a reagent for sulfhydryl determinations,<sup>14</sup> with a sensitivity to 0.1 mM.<sup>55</sup> The adsorption spectrum has a maximum at approximately 300 nm, which disappears as NEM reacts with sulfhydryl groups.

### **Preparation Instructions**

NEM gives a clear solution in ethanol at 50 mg/mL. NEM dissolves in water (>50 mg in 4 mL); however, aqueous solutions are unstable. The rate of hydrolysis is pseudo-first order and significantly dependent on pH. The half-lives of solutions at different pH were reported.<sup>52</sup>

<b>pH</b>	<b>Half-life (hrs)</b>
6.5	160
7.0	48
7.5	9
8.0	1.7
8.25	0.7
8.5	0.33

Preparation of fresh solutions for each usage is recommended. The solution concentration should be essentially constant for approximately three hours at room temperature at or below pH 7.0.<sup>53</sup>

## **Leupeptin hemisulfate**

Product Number **L2884**

Synonym: Acetyl-Leu-Leu-Arg-al

### **Product Description**

Leupeptin is a reversible, competitive inhibitor of serine and thiol proteases.<sup>57</sup> It has been reported to inhibit calpain,<sup>58</sup> cathepsin B,<sup>59</sup> cathepsins H and L,<sup>60</sup> and trypsin.<sup>61</sup>

<b>Enzyme</b>	<b>Inhibition</b>
Acrosin	$K_i = 210 \text{ nM}^{62}$
Calpains	$K_i = 10\text{-}430 \text{ nM}^{63}$
Cathepsin B	$K_i = 4.1 \text{ nM}^{64}$
Chymotrypsin	$K_i = 1.1 \text{ mM}^{65}$
Histolysin	$K_i = 43 \text{ nM}^{66}$
Plasmin	$K_i = 3.4 \text{ mM}^{67}$
Trypsin	$K_i = 3.5 \text{ nM}^{67}$

### **Preparation Instructions**

Salts of leupeptin are reported to be soluble in water, ethanol, acetic acid, and DMF. A 10 mM aqueous solution is stable for a week at 4 °C,<sup>56</sup> and for at least 6 months at -20 °C as frozen aliquots. Typical working concentrations are in the range of 10 to 100 μM.<sup>60</sup>

The primary mechanism of inactivation is racemization of the L-arginal, as the D-arginal isomer is totally inactive. If the aldehyde is oxidized, but retains its L-configuration, the resulting compound does have some inhibitory activity.<sup>14</sup>

## **Pepstatin A**

From microbial source

Sigma Code **P5318**

Synonyms: Isovaleryl-Val-Val-Sta-Ala-Sta Sta (statine) is (3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid

### **Product Description**

Pepstatin A is an inhibitor of acid proteases (aspartyl peptidases). It forms a 1:1 complex with proteases such as pepsin,<sup>68,69</sup> renin,<sup>68,69</sup> cathepsin D,<sup>68,69</sup> bovine chymosin,<sup>69</sup> and protease B (*Aspergillus niger*).<sup>70</sup> The inhibitor is highly selective<sup>71</sup> and does not inhibit thiol proteases, neutral proteases, or serine proteases. Solubilized  $\gamma$ -secretase<sup>72</sup> and retroviral protease<sup>73</sup> are also inhibited by Pepstatin A. It has been used to characterize proteases from several sources.<sup>74,75</sup>

### **Preparation Instructions**

Pepstatin can be dissolved at 1 mg/mL in 10% (v/v) acetic acid in methanol (9:1 methanol:acetic acid). The inclusion of acetic acid may be necessary to dissolve this peptide in methanol or DMSO. It has been dissolved at 1 to 2 mg/mL in ethanol, but heat may be required for complete dissolution. Solutions of Pepstatin A can be heated as high as 60 °C without any decomposition of the peptide.

Stock solutions at 1 mg/mL are stable at least a week at 4 °C. A 1 mM solution in methanol or DMSO is stable for months at -20 °C. If solutions become darker yellow, the reagent is hydrolyzing.

A working concentration of 1  $\mu$ M<sup>68</sup> is stable for at least one day at room temperature.

## **Phosphoramidon disodium salt**

Product Number **R7385**

Synonym: N-( $\alpha$ -Rhamnopyranosyloxyhydroxy-phosphinyl)-Leu-Trp disodium salt

### **Product Description**

Phosphoramidon is a strong inhibitor of many bacterial metalloendoproteases, thermolysin, and elastase, but a weak inhibitor of collagenase.<sup>77,78,79</sup> It does not inhibit trypsin, papain, chymotrypsin, and pepsin.<sup>76,79</sup> Mild hydrolysis of phosphoramidon yields phosphoryl-L-leucyl-L-tryptophan which is more active than phosphoramidon.<sup>76</sup>

### **Preparation Instructions**

Solubility testing at 10 mg/mL in water yields a clear, colorless to light yellow-green solution. The product is also soluble in methanol and DMSO; less soluble in ethanol and ethyl acetate; insoluble in benzene, hexane, and chloroform.<sup>76</sup>

Solutions can be stored in aliquots at -20 °C with an expected shelf life of at least one month.

**Trypsin inhibitor from *Glycine max* (Soybean)**  
**Type I-S**

Product Number **T9003**

Synonyms: Kunitz Trypsin Inhibitor; TI<sup>a</sup>; STI

**Product Description**

Soybean trypsin inhibitor inhibits trypsin<sup>80</sup> and to a lesser extent chymotrypsin and plasmin. It forms a 1:1 stoichiometric complex with trypsin. Upon formation of this complex, trypsin may cleave a single arginine-isoleucine bond in the inhibitor.<sup>81,82</sup> Dissociation of this complex may yield the modified form or the native inhibitor.<sup>83</sup> At the optimal pH for trypsin binding (pH 8.0), the association constant is  $> 10 \times 10^8$ .<sup>84</sup>

**Preparation Instructions**

Trypsin inhibitor is soluble in water and phosphate buffers at concentrations of 10 mg/mL or higher. Solutions at higher concentrations may be hazy and have a yellow to amber color.

A 1% sterile-filtered solution stored for 3.5 years at 2-8 °C showed no loss in trypsin inhibition activity. Solutions are stable in frozen aliquots at -20 °C, but freeze-thaw should be avoided. This protein is reversibly denatured by short heating to 80 °C and irreversibly inhibited by heating to 90 °C.<sup>80</sup>

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