

Product Information

Trypsin inhibitor from *Glycine max* (soybean) Cell Culture Tested

Product Number **T 6522**
Storage Temperature 2-8 °C

Product Description

CAS Number: 9035-81-8
Extinction Coefficient: $E^{1\%} = 9.94$ (280 nm,
pH 7.6 buffer)
pI: 4.5¹
Synonyms: Kunitz Trypsin Inhibitor, Ti^{a1}, STI, and
SBT1

This product is cell culture tested and is appropriate for use in cell culture applications. It is extensively dialyzed against water. After dialysis, sodium phosphate buffer, pH 7.6, is added, and the inhibitor is lyophilized. The final product consists of about 90% protein and 10% sodium phosphate buffer salts (by mass).

Soybean trypsin inhibitor was first isolated by Kunitz.² Several other related inhibitors are also found in soybeans.³ Trypsin inhibitor from soybeans is a monomeric protein containing 181 amino acid residues in a single polypeptide chain crosslinked by two disulfide bridges.^{4,5,6} The molecular weight determined from the amino acid sequence is 20.1 kDa.

Soybean trypsin inhibitor inhibits trypsin, and to a lesser extent chymotrypsin⁸ and plasmin.⁹ Soybean trypsin inhibitor will also inhibit proteases with mechanisms similar to trypsin. This product will also inhibit plasma kallikrein and coagulation Factor X_a. However, this product will not inhibit metalloproteases, tissue-based kallikrein, acid proteases, or thio proteases.

Soybean trypsin inhibitor forms a 1:1 stoichiometric complex with the protease active site. Upon formation of this complex, trypsin may cleave a single arginine-isoleucine bond on the inhibitor.^{10,11} Inhibition is both reversible and pH dependent. Dissociation of this complex may yield a modified or native form of the inhibitor.¹² The optimal pH for trypsin binding is 8.0 with an association constant of greater than 10^9 at pH 8.0 and (1.5×10^3 to 2.6×10^4) at pH 3.6 to 4.4.¹³

Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

Preparation Instructions

Trypsin inhibitor is soluble in water and phosphate buffers at 10 mg/ml. It is soluble in balanced salt solutions (1 mg/ml) and in serum-free media. Solutions at concentrations higher than 10 mg/ml may be hazy and have a yellow to amber color.

Storage/Stability

A 10 mg/ml sterile-filtered solution stored for greater than 3 years at 2-8 °C showed no loss in trypsin inhibition activity. Solutions are stable in frozen aliquots at -20 °C, but freeze-thaw cycles should be avoided. This protein is reversibly denatured by short heating to 80 °C and irreversibly inhibited by heating to 90 °C.³

Procedure

After trypsinizing cells, resuspend cells in 1 ml trypsin inhibitor solution (1 mg/ml) for every ml of trypsin solution used for dissociation. Centrifuge the cell suspension at 1000 rpm (200 x g) for 5 minutes. A cell pellet should form. Remove as much of the trypsin inhibitor as possible and resuspend the pellet in serum-free medium. Culture cells as desired.

References

1. Kunitz, M., J. Gen. Physiol., **30**, 311 (1947).
2. Kunitz, M., Science, **101**, 668 (1945).
3. Steiner, R. F., and Frattali, V. J., Agr. Food Chem., **17**, 513 (1969).
4. Kim, S. H., et al., Comparative study on amino acid sequences of Kunitz-type soybean trypsin inhibitors, Tia, Tib, and Tic. J. Biochem. (Tokyo), **98(2)**, 435-448 (1985).
5. Steiner, R. F., Biochim. Biophys. Acta, **100**, 111 (1965).

6. Koide, T., and Ikenaka, T., Studies on soybean trypsin inhibitors. 1. Fragmentation of soybean trypsin inhibitor (Kunitz) by limited proteolysis and by chemical cleavage. *Eur. J. Biochem.*, **32(3)**, 401-407 (1973).
7. Wu, Y. V., and Scheraga, H. A., *Biochemistry*, **1**, 698 (1962).
8. De Vonis Bidlingmeyer, U., et al., Identity of the tryptic and alpha-chymotryptic reactive sites on soybean trypsin inhibitor (Kunitz). *Biochemistry*, **11(17)**, 3303-3310 (1972).
9. Nanninga, L. B., and Guest, M. M., *Arch, Biochem. Biophys.*, **108**, 542 (1964).
10. Ozawa, K., and Laskowski, M. Jr., The reactive site of trypsin inhibitors. *J. Biol. Chem.*, **241(17)**, 3955-3961 (1966).
11. Finkenstadt, W. R., and Laskowski, M. Jr., Resynthesis by trypsin of the cleaved peptide bond in modified soybean trypsin inhibitor. *J. Biol. Chem.*, **242(4)**, 771-773 (1967).
12. Finkenstadt, W. R., and Laskowski, M. Jr., *J. Biol. Chem.*, **240**, 962 (1965).
13. *Advances in Protein Chemistry*, vol. 9, Laskowski, M., Academic Press (New York, NY: 1954), p. 226.

ALF/RXR 2/03

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.