SIGMAFAST™ Protease Inhibitor Tablet
For General Use

Catalog Number S8820
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
Crude cell extracts contain a number of endogenous enzymes, such as proteases, which are capable of degrading the proteins present in the sample. The best way to improve the yield of intact proteins is to add inhibitors of the proteases to the source material.

The SIGMAFAST™ Protease Inhibitor Tablet is a mixture of water soluble protease inhibitors with a broad specificity for the inhibition of serine, cysteine, and metalloproteases. The tablets have been optimized to inhibit a wide range of different proteases and are recommended for general applications.

The SIGMAFAST Protease Inhibitor Tablet is not recommended for use with HIS-Select® products or any other immobilized metal affinity chromatography (IMAC) products. The tablets contain EDTA, a metal chelator, which may remove metal ions from these affinity products. The suggested protease inhibitor for the HIS-Select products is Protease Inhibitor Cocktail For Use in Purification of Histidine-tagged proteins (Catalog Number P8849).

Components
Each tablet can be used to prepare 100 mL of 1× protease inhibitor solution, which contains the following inhibitor concentrations:

- AEBSF 2 mM
- EDTA 1 mM
- Bestatin 130 µM
- E-64 14 µM
- Leupeptin 1 µM
- Aprotinin 0.3 µM

The individual components of the protease inhibitor tablet have specific inhibitory properties. A description of each inhibitor is given below.

- AEBSF (4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride) inhibits serine proteases, such as trypsin and chymotrypsin.¹⁴
- EDTA (Ethylenediaminetetraacetic acid) inhibits metalloproteases.⁵
- Bestatin inhibits aminopeptidases, such as leucine and alanyl aminopeptidases.⁶
- E-64 (N-(trans-Epoxysuccinyl)-L-leucine 4-guanidinobutylamide) inhibits cysteine proteases, such as calpain, papain, cathepsin B, and cathepsin L.⁷⁻¹⁵
- Leupeptin inhibits both serine and cysteine proteases, such as plasmin, trypsinogen, urokinase, and kallikrein.¹⁶
- Aprotinin inhibits trypsin and human leukocyte elastase.¹⁷

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
One tablet generates 100 mL of protease inhibitor solution. Each tablet can be reconstituted in either water or buffer, and may be prepared as a 10× concentrate and diluted as needed. Concentrations greater than 1× may appear hazy. This will not affect the performance of the protease inhibitors. Mix the 10× concentrate until uniformly suspended. Dilute as appropriate.
Storage/Stability
The tablets are stable as supplied for at least 4 years at 2–8 °C. The reconstituted protease inhibitor solution (1× or 10×) is stable for at least 2 weeks at 2–8 °C. It is not recommended to freeze reconstituted solutions (1× or 10×), as some material may precipitate.

Procedure
One mL of the 1× protease inhibitor solution is recommended for the inhibition of proteases equivalent to 1 mg of USP pancreatin.

One tablet is recommended for the inhibition of proteases present in a maximum of 20 g of cell extract. Since not all organisms contain the same amount of endogenous proteases it may sometimes be necessary to increase the concentration of inhibitors.

References

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