Product Information

SIGMA*FAST*™ Protease Inhibitor Cocktail Tablets, EDTA-Free

for use in purification of Histidine-tagged proteins

S8830

Product Description

Crude cell extracts contain many endogenous enzymes, such as proteases, which can degrade the proteins present in the sample. The best way to preserve the integrity of the proteins is to add a broad spectrum of protease inhibitors tailored to the sample and task.

The Sigma*FAST*™ Protease Inhibitor Cocktail Tablet, EDTA-Free is a mixture of protease inhibitors with a broad specificity for the inhibition of serine, cysteine, aspartic, and metalloproteases. The tablets have been formulated to be compatible with sample processing by immobilized metal affinity chromatography (IMAC) such as used for the purification of histidine-tagged (His-tagged) proteins. The tablets contain no strong metal chelators, such as EDTA, which might interfere with IMAC processing. Finally, the tablets contain a unique, proprietary formulation which includes a pepstatin A salt to provide additional protection not normally obtained in a tablet format. The broad protection afforded allows use of these tablets to purify His-tagged proteins and as a general-purpose protease inhibitor cocktail.

Several theses[1] and dissertations[2-10] have cited use of product S8830 in their protocols.

Components

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

- AEBSF: 2 mM
- Phosphoramidon: 1 µM
- Bestatin: 130 µM
- E-64: 14 µM
- Leupeptin: 1 µM
- Aprotinin: 0.2 µM
- Pepstatin A: 10 µM

Specific inhibitory properties of the components are:

- AEBSF [4-(2-Aminoethyl)benzenesulfonyl fluoridehydrochloride]: serine proteases, such as trypsin, chymotrypsin, plasmin, kallikrein, and thrombin
- Bestatin hydrochloride: aminopeptidases, such as leucine aminopeptidase and alanyl aminopeptidase[11-14]
- Leupeptin: serine and cysteine proteases, such as trypsin, plasmin, trypsinogen, urokinase, and kallikrein[13]
- E-64 [*N*-(trans-Epoxysuccinyl)-L-leucine 4-guanidinobutylamide]: cysteine proteases, such as calpain, papain, cathepsin B, and cathepsin L[11]
- Aprotinin: serine protease, such as chymotrypsin, trypsin, and elastase
- Pepstatin A: acid proteases, such as pepsin, renin, and cathepsin D, and many microbial aspartic proteases
- Phosphoramidon disodium salt: thermolysin and collagenase

Precautions and Disclaimer

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.

Storage/Stability

The tablets are stable as supplied for at least 2 years at 2-8 °C. The reconstituted protease inhibitor solution (1× or 10×) is stable for at least 2 weeks at 2-8 °C.

Preparation Instructions

One tablet generates 100 mL of 1× protease inhibitor solution. Each tablet can be reconstituted in either water or extraction/lysis buffer.

A tablet may also be used to prepare a 10× protease inhibitor solution, which can be diluted as needed. Concentrations greater than 1× may appear slightly hazy. This will not affect the performance of the protease inhibitors. Mix the 10× protease inhibitor solution (15 minutes) until uniformly suspended, and then dilute as appropriate.

Procedure

- One tablet is recommended for the inhibition of the proteases present in a maximum of 20 g of cell mass in 100 mL of extract.
- The inhibitor mixture may be dissolved directly in the extraction reagent or added from the 10× protease inhibitor solution immediately following extraction.
- Since not all organisms contain the same amounts of endogenous proteases, it may sometimes be necessary to increase the concentration of inhibitors.

References

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