

## Product Information

# SIGMAFAST™ Protease Inhibitor Tablets

For General Use

**S8820**

## 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 S8820 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 S8820 tablets have been optimized to inhibit a wide range of different proteases and are recommended for general use.

The S8820 SIGMAFAST™ Protease Inhibitor Tablets are **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. For HIS-Select® products, suggested protease inhibitor cocktails are:

- Cat. No. S8830: SIGMAFAST™ Protease Inhibitor Cocktail Tablets, EDTA-Free
- Cat. No. P8849: Protease Inhibitor Cocktail for use in purification of Histidine-tagged proteins
- Cat. No. PIC0004: ReadyShield® Protease Inhibitor Cocktail for use in purification of Histidine-tagged proteins, Non-freezing solution

Several dissertations<sup>1-4</sup> have cited use of product S8820 in their protocols.

## Components

Each S8820 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

Specific inhibitory properties of the components are:

- AEBSF, 4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride: inhibits serine proteases, such as trypsin and chymotrypsin<sup>5-8</sup>
- EDTA (Ethylenediaminetetraacetic acid): inhibits metalloproteases<sup>9</sup>
- Bestatin: inhibits aminopeptidases, such as leucine and alanyl aminopeptidases<sup>10</sup>
- E-64, N-(*trans*-Epoxy succinyl)-L-leucine 4-guanidinobutylamide: inhibits cysteine proteases, such as calpain, papain, cathepsin B, and cathepsin L<sup>11-19</sup>
- Leupeptin: inhibits both serine proteases and cysteine proteases, such as plasmin, trypsinogen, urokinase, and kallikrein<sup>20</sup>
- Aprotinin: inhibits trypsin and human leukocyte elastase<sup>21</sup>

## 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 4 years at 2-8 °C.
- The reconstituted S8820 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.

## Preparation Instructions

One S8820 tablet generates 100 mL of protease inhibitor solution. Each tablet can be reconstituted in either water or buffer.

The solution may be prepared as a 10× concentrate (10 mL volume) and diluted as needed.

Concentrations greater than 1× may appear hazy. This will not affect the performance of the protease inhibitors. Mix such >1× concentrates until uniformly suspended.

## 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 amounts of endogenous proteases, it may sometimes be necessary to increase the concentration of inhibitors.

## References

1. Borges Vigil, Fabio Antonio, "The roles of endogenous Calcium/Calmodulin-dependent kinase II inhibitors in learning and memory". King's College London, Ph.D. dissertation, p. 176 (2015).
2. Varghese, Robin, "Novel Prognostic Markers and Therapeutic Targets for Glioblastoma". Virginia Polytechnic Institute and State University, Ph.D. dissertation, p. 21 (2016).
3. Ajina, Reham S., "Identifying molecular determinants of immune rejection in pancreatic cancer". Georgetown University, Ph.D. dissertation, p. 110 (2020).
4. Rabe, Brian Anthony, "Investigation of the Multiple Roles of Notch Signaling in Embryonic Retinal Development Using Novel High-Throughput Techniques". Harvard University, Ph.D. dissertation, p. 145 (2020).
5. Mintz, G.R., *BioPharm*, **6(2)**, 34-38 (1993).
6. Markwardt, F. *et al.*, *Thrombosis Res.*, **2(4)**, 343-348 (1973).
7. Lawson, W.B. *et al.*, *Folia Haematol. Int. Mag. Klin. Morphol. Blutforsch.*, **109(1)**, 52-60 (1982).
8. Markwardt, F. *et al.*, *Biochem. Pharmacol.*, **23(16)**, 2247-2256 (1974).
9. Beynon, R., and Bond, J.S. (eds.), *Proteolytic Enzymes, A Practical Approach*, 2<sup>nd</sup> edition. Oxford University Press (Oxford, UK), p. 322 (2001).
10. Fritz, H., and Wunderer, G., *Arzneimittelforschung*, **33(4)**, 479-494 (1983).
11. Aoyagi, T., and Umezawa, H., *Acta Biol. Med. Ger.*, **40(10-11)**, 1523-1529 (1981).

12. Hanada, K. *et al.*, *Agric. Biol. Chem.*, **42(3)**, 523-528 (1978).
13. Hashida, S. *et al.*, *J. Biochem.*, **88(6)**, 1805-1811 (1980).
14. Barrett, A.J. *et al.*, *Biochem. J.*, **201(1)**, 189-198 (1982).
15. Sugita, H. *et al.*, *J. Biochem.*, **87(1)**, 339-341 (1980).
16. Katunuma, N. and Kominami, E., *Methods Enzymol.*, **251**, 382-397 (1995).
17. Barrett, A.J. and Kirschke, H., *Methods Enzymol.*, **80**, 535-561 (1981).
18. Inubushi, T. *et al.*, *J. Biochem.*, **116(2)**, 282-284 (1994).
19. Nomura, T. *et al.*, *Biochem. Biophys. Res. Commun.*, **228(3)**, 792-796 (1996).
20. Hanada, K. *et al.*, *Agric. Biol. Chem.*, **42(3)**, 537-541 (1978).
21. Umezawa, H., *Methods Enzymol.*, **45**, 678-695 (1976).

## Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

## Technical Assistance

Visit the tech service page at [SigmaAldrich.com/techservice](https://SigmaAldrich.com/techservice).

## Standard Warranty

The applicable warranty for the products listed in this publication may be found at [SigmaAldrich.com/terms](https://SigmaAldrich.com/terms).

## Contact Information

For the location of the office nearest you, go to [SigmaAldrich.com/offices](https://SigmaAldrich.com/offices).

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

MilliporeSigma, and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.

© 2022 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

S8820dat Rev 06/22 ASC,GCY,MAM

**MILLIPORE  
SIGMA**