Product Information

Protease Inhibitor Cocktail

For use in purification of Histidine-tagged proteins, DMSO solution

P8849

Product Description

Crude cell extracts contain various endogenous enzymes, such as proteases and phosphatases, which can degrade proteins in the extracts. The best way to increase the yield of intact proteins is to add inhibitors of those enzymes known to be present.

This protease inhibitor cocktail has a broad specificity for the inhibition of serine, cysteine, acid, and thermolysin-like proteases, and aminopeptidases. P8849 particularly omits chelators (such as EDTA), to optimize its use for purification of histidine-tagged (His-tagged) proteins.[1]

P8849 is supplied as a solution in DMSO. P8849 contains five protease inhibitors, with the following specific inhibitory properties:

- AEBSF [4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride]: serine proteases, such as trypsin, chymotrypsin, plasmin, kallikrein, and thrombin
- Bestatin hydrochloride: aminopeptidases, such as leucine aminopeptidase and alanyl aminopeptidase[2–5]
- E-64 [*N*-(trans-Epoxysuccinyl)-L-leucine 4-guanidinobutylamide]: cysteine proteases, such as calpain, papain, cathepsin B, and cathepsin L
- Pepstatin A: acid proteases, such as pepsin, rennin, and cathepsin D, and many microbial aspartic proteases
- Phosphoramidon disodium salt: thermolysin and collagenase

Several theses[6-9] and dissertations[10-22] have cited use of product P8849 in their protocols.

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

Store the product at –20 °C. This product has a recommended retest date of 4 years, when stored under defined conditions, and the container has remained unopened.

Usage

One mL of P8849 cocktail solution is recommended for the inhibition of endogenous enzymes found in 100 mL of lysate from 20 g (wet weight) of *E. coli* cells or 10 g (wet weight) of baculovirus-infected cells. The *E. coli* cells were grown on LB medium. An extract of baculovirus-infected *Spodoptera frugiperda* pupal ovary cells was also tested.

Note: Not all lysates contain the same levels of endogenous proteases. It may be necessary to adjust the volume of cocktail required.

References

1. Bornhorst, J.A., and Falke, J.J., *Methods Enzymol.*, **326**, 245-254 (2000).
2. Umezawa, H., *Ann. Rev. Microbiol.*, **36**, 75-99 (1982).
3. Aoyagi, T. *et al.*, *Biochem. Int.*, **9(4)**, 405-411 (1984).
4. Aoyagi, T., and Umezawa, H., *Acta Biol. Med. Ger.*, **40(10-11),** 1523-1529 (1981).
5. Mumford, R.A. *et al.*, *Biochem. Biophys. Res. Comm.*, **103(2)**, 565-572 (1981).
6. Beasley, Charles Britton, Jr., "The Role of Sulfatide in Alzheimer's Disease". Virginia Commonwealth University, M.S. thesis, p. 25 (2006).
7. Kotun, Allen M., "Biochemical Analysis of Cell Division Protein Complexes in *Streptomyces coelicolor*". Duquesne University, M.S. thesis, pp. 30, 31 (2007).

8. Chan, Philemon Huei-Han, "Construction of CNFY-CNF1 Chimera Protein for Study of Differential pH Dependent Activity of CNF Proteins". University of Illinois at Urbana-Champaign, M.S. thesis, p. 17 (2014).
9. Castillo, Allan, "The Role of Critical Ion Pairs in the Evolution of a Novel Enzyme in Tomato". Duke University, M.S. thesis, p. 12 (2018).
10. Wang, Dongye, "Ensemble fluorescence resonance energy transfer analysis of RNA polymerase clamp conformation". Rutgers, The State University of New Jersey, Ph.D. dissertation, pp. 64 (2008).
11. Savinova, Olga V., "p105 and p100 proteins function as the core of heterogeneous NF-kappaB complexes". University of California San Diego, Ph.D. dissertation, pp. 11, 32 (2009).
12. Mo, Allison Huei-Juin, "Functional analysis of YneA, an SOS-induced inhibitor of cell division in *Bacillus subtilis*". Stanford University, Ph.D. dissertation, pp. 58, 59 (2010).
13. Berry, Joel Dallas, "The Final Step in Phage Lysis: The Role of the Rz-Rz1 Spanin Complex in the Disruption of the Outer Membrane". Texas A&M University, Ph.D. dissertation, pp. 78, 102 (2010).
14. Forster, Brian Michael, "Regulation of the Metalloprotease 0f *Listeria Monocytogenes* During Intracellular Infection". Cornell University, Ph.D. dissertation, p. 117 (2011).
15. Boswell, Leaf Chandra, "Group 3 late Embryogenesis abundant proteins from embryos of *Artemia francisana*: molecular characteristics, expression and function". Louisiana State University, Ph.D. dissertation, p. 22 (2013).
16. Martin, Kyle Benjamin, "Identification of altered Ras signaling and intermediate filament hyperphosphorylation in giant axonal neuropathy". Indiana University, Ph.D. dissertation, p. 43 (2015).
17. Cai, Jing, "Superior to one of Glass: Natural Gradient index Lenses via Patchy Particle Self-Assembly". University of Pennsylvania, Ph.D. dissertation, pp. 36, 44, 48, 76 (2015).
18. Nicholes, Nathan, "Improved Methods for the Development and Adaptation of Protein Switches". Johns Hopkins University, Ph.D. dissertation, pp. 38, 39, 57, 78, 79 (2015).
19. Low, Darryl Weijun, "Synaptic Vesicle Protein 2a-Dependent Function and Dysfunction at the Presynapse". University of Edinburgh, Ph.D. dissertation, pp. 72-74 (2017).
20. Arrington, Megan Elizabeth, "Modes of Regulation of RAC GTPases". University of North Carolina at Chapel Hill, Ph.D. dissertation, p. 105 (2019).
21. Tripler, Therese N., "Conservation and Divergence Between P22-like Bacteriophages Coat Protein's I-domains and Procapsid-like Particles". University of Connecticut, Ph.D. dissertation, pp. 16, 18 (2019).
22. Chandan, Naincy, "Identification and Characterization of G Protein Signaling Networks by Proximity Labeling-Coupled Proteomics". University of Michigan, Ph.D. dissertation, pp. 56, 88 (2021).

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