

Product Information

Protease Inhibitor Cocktail

For use in tissue culture media

P1860

Product Description

This protease inhibitor cocktail is designed for use in tissue culture media and is a mixture of protease inhibitors with a broad specificity for serine, cysteine, and acid proteases, and aminopeptidases. P1860 should be used as a supplement to tissue culture media, to prevent the degradation of secreted proteins from the cultured tissue.

P1860 was found to be non-toxic to the following cell lines after 48 hours exposure:

- A431, CHO, COS, HepG2, and HeLa adherent cell lines
- Jurkat and HL-60 cell lines grown in suspension

The inhibitors in P1860 are as follows, with respective specific inhibitor targets and target classes of each inhibitor listed:

- Aprotinin: serine proteases, such as trypsin, chymotrypsin, plasmin, and kallikrein; human leukocyte elastase, but not pancreatic elastase
- Bestatin hydrochloride: aminopeptidases, such as leucine aminopeptidase and alanyl aminopeptidase¹⁻⁴
- E-64, *N*-(trans-Epoxy succinyl)-L-leucine 4-guanidinobutylamide: cysteine proteases, such as calpain, papain, cathepsin B, and cathepsin L
- Leupeptin hemisulfate salt: both serine proteases and cysteine proteases, plasmin, trypsin, papain, and cathepsin B
- Pepstatin A: acid proteases, such as pepsin, renin and cathepsin D, and many microbial aspartic proteases

Several theses⁵⁻⁸ and dissertations⁹⁻²¹ have cited use of product P1860 in their protocols.

P1860 is supplied as a solution in DMSO, and was prepared using sterile-filtered DMSO (Hybri-Max™).

Storage/Stability

Store the cocktail at -20 °C. The product, as supplied, is stable for 4 years when stored at -20 °C, 8 months at 2-8 °C, and 2 months at room temperature.

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.

Recommended Usage

It is recommended to determine the dilution appropriate for a specific cell line. This testing should begin with at least a 200-fold dilution, because DMSO concentrations of > 0.5% may be deleterious to cell growth. Further dilutions, such as 400-fold or 800-fold, may be necessary, since cell lines will differ in their sensitivity to this protease inhibitor cocktail.

For cell toxicity testing of P1860:

- A 200-fold dilution was used with the A431 and COS cell lines.
- An 800-fold dilution was used with the CHO, HeLa, HepG2, Jurkat, and HL-60 cell lines.

The cocktail will remain effective for up to 48 hours in the medium. After this period, the medium should be replaced with freshly prepared medium containing the cocktail.

References

1. Umezawa, H., *Ann. Rev. Microbiol.*, **36**, 75-99 (1982).
2. Aoyagi, T. *et al.*, *Biochem. Int.*, **9(4)**, 405-411 (1984).
3. Aoyagi, T., and Umezawa, H., *Acta Biol. Med. Ger.*, **40(10-11)**, 1523-1529 (1981).
4. Mumford, R.A. *et al.*, *Biochem. Biophys. Res. Comm.*, **103(2)**, 565-572 (1981).

5. Wei, Wangzhi, "The Role of Alternatively spliced Fibroblast Growth Factor Receptor 2 Isoforms in Breast Cancer". University of Toronto, M.Sc. thesis, p. 27 (2011).
6. Requena, Martin Daniel, "A cell culture model of MRPS2-related Cutis laxa". University of Pittsburgh, M.S. thesis, p. 8 (2017).
7. Arras, Wout, "Gene insertion and excision: a step towards the reversible immortalisation of human corneal endothelial cells". Universiteit Antwerpen, M.Sc. thesis, p. 39 (2020).
8. Pham, Diana Minh, "Modulation of TGFβ1-induced Fibroblast-to-Myofibroblast Transition in response to Prostaglandin E2 Production by Human Rhinovirus-Infected Airway Epithelial Cells". University of Alberta, M.Sc. thesis, p. 32 (2020).
9. Mathieson, William, "A Proteomic and Functional Study of the *Schistosoma mansoni* Egg". University of York, Ph.D. dissertation, pp. 13, 66 (2007).
10. Gray, Reginald Courtney, "Regulation of interferon αβ induction and dendritic cell function by CpG oligodeoxynucleotides". Case Western Reserve University, Ph.D. dissertation, pp. 87, 88 (2008).
11. Morad, Samy Abd EL-Raouf Fahim Khalafalla, "Pharmacological Studies of a Novel Inhibitor of the Mammalian Target of Rapamycin (mTOR) Signaling Pathway". Ludwig Maximilians University of Munich, Dr. vet. med. dissertation, pp. 43, 45 (2010).
12. Ahmadabadi, Seyed Rouhollah Mousavizadeh, "Evaluation of the Role of ANGPTL4 in Tendon Vascularization". University of British Columbia, Ph.D. dissertation, p. 120 (2015).
13. Bottrell, Alyssa, "The role of PDGF C and its splice variant in breast cancer". Wayne State University, Ph.D. dissertation, p. 35 (2015).
14. Cox, April Ann, "Estrogen Nanoparticles in Spinal Cord Injury". Medical University of South Carolina, Ph.D. dissertation, p. 36 (2015).
15. Subhashini, Nidhi, "Dlk1 Membrane-to-Nuclear Signalling During Motor Neuron Functional Diversification". Georg August University Göttingen, Dr. rer. nat. dissertation, p. 33 (2016).
16. Barutcu, Seda, "Role of JIP1-JNK Signaling in Beta-Cell Function and Autophagy". University of Massachusetts Graduate School of Biomedical Sciences, Worcester, Ph.D. dissertation, p. 102 (2018).
17. Prodanović, Danica, "Novel insights into mechanisms of glucocorticoid actions and sensitivity in the airway epithelium". University of Melbourne, Ph.D. dissertation, p. 60 (2018).
18. Saraiva, Raúl G., "*Kosakonia* & *Chromobacterium* vs. malaria & dengue – insights into antipathogenic strategies of mosquito midgut bacteria". Johns Hopkins University, Ph.D. dissertation, p. 68 (2016).
19. Birch, Gavin Peter, "The Development of SmartProbes for the Optical Imaging of Pulmonary Inflammation". University of Edinburgh, Ph.D. dissertation, p. 159 (2019).
20. Speer, Shannon Leigh, "Protein complex stability in living cells". University of North Carolina at Chapel Hill, Ph.D. dissertation, p. 50 (2020).
21. Galvin, Sam George, "A peptidomic investigation into enteroendocrine cells and islets of humans and mice". University of Cambridge, Ph.D. dissertation, p. 95 (2021).

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