Product Information

ReadyShield™ Protease Inhibitor Cocktail

For Use in Tissue Culture Media

PIC0006

Product Description

Proteins secreted by cells grown in culture can be modified and degraded by proteases that are also secreted by the same cells. The activity of such proteases is even more pronounce when cells are grown in serum free media. This proteolytic activity can reduce the production level of secreted proteins or modify the results of secreted proteome analysis.

This ReadyShield™ Protease Inhibitor Cocktail is a mixture of protease inhibitors with a broad specificity for the inhibition of serine, cysteine, aspartic proteases and aminopeptidases. It is designed to be used as a supplement to tissue culture medium for the prevention of secreted proteins degradation. The product has been found to be non-toxic after a 48 hours exposure to the following adherent cell lines: A431, CHO, COS, HepG2, and HeLa, and to following non-adherent cell lines: Jurkat and HL-60.

This cocktail is supplied as a ready-to-use solution using a proprietary non-freezing formulation.

ReadyShield™ Protease Inhibitor Cocktail for use in tissue culture media is a non-freezing formulation that contains the same inhibitors as is the DMSO based cocktail P1860.

This cocktail contains five protease inhibitors, with the following specific inhibitory properties:

- AEBSF or 4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride: serine proteases, e.g., trypsin, chymotrypsin, plasmin, kallikrein, and thrombin
- Bestatin hydrochloride: aminopeptidases, e.g., leucine aminopeptidase and alanyl aminopeptidase [1-4]
- E-64 or N-(trans-Epoxysuccinyl)-L-leucine 4-guanidinobutylamide: cysteine proteases, e.g., calpain, papain, cathepsin B, and cathepsin L
- Pepstatin A: acid proteases, e.g., pepsin, rennin, and cathepsin D, and many microbial aspartic proteases
- Phosphoramidon disodium salt: thermolysin and collagenase

Precautions and Disclaimer

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

Storage/Stability

The cocktail is shipped on wet ice and storage at –20 °C is recommended. The product, as supplied, is stable for two years. For short time periods the product can be stored at 2-8 °C.

Procedure

It is recommended to determine the appropriate dilution for each specific cell line. This testing should begin with a dilution of at least a 200-fold to prevent deleterious effect of the solvent to cell growth. Further dilutions, of 400-fold or 800-fold may be necessary, since various cell lines will differ in their sensitivity to this protease inhibitor cocktail.

For cell toxicity testing at Sigma, a 200-fold dilution was used with the A431 and COS cell lines and a 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).

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