



## Product Information

### Protease Inhibitor Cocktail

For use in Tissue Culture Media

Product Number **P 1860**

Storage Temperature  $-20^{\circ}\text{C}$

#### Product Description

This is a mixture of protease inhibitors with a broad specificity for the inhibition of serine, cysteine, aspartic and aminopeptidases.

This product should be used as a supplement to tissue culture medium to prevent the degradation of secreted proteins. After 48 hours exposure the product was found to be non-toxic to the following: A431, CHO, COS, HepG2, and HeLa adherent cell lines, and to Jurkat and HL-60 cell lines grown in suspension.

#### Components

The individual components of this proprietary formulation have specific inhibitory properties. A description of each inhibitor is given below.

Aprotinin (Product Code A 1153) inhibits serine proteases, such as trypsin, chymotrypsin, plasmin, trypsinogen, urokinase, and kallikrein. Aprotinin inhibits human leukocyte elastase, but not pancreatic elastase.

Bestatin (Product Code B 8385) inhibits aminopeptidases, such as leucine aminopeptidase and alanyl aminopeptidase.<sup>1,2,3,4</sup>

E-64 [trans-epoxysuccinyl-L-leucylamido(4-guanidino)butane] (Product Code E 3132) inhibits cysteine proteases, such as calpain, papain, cathepsin B, and cathepsin L.

Leupeptin (Product Code L 2884) inhibits both serine and cysteine proteases, such as calpain, trypsin, papain, and cathepsin B.

Pepstatin A (Product Code P 4265) inhibits acid proteases, such as pepsin (human or porcine), renin, cathepsin D, chymosin (bovine rennin), and protease B (*Aspergillus niger*).

#### 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.

#### Preparation Instructions

The cocktail is supplied as a clear, colorless solution in dimethyl sulfoxide (DMSO). It is prepared with sterile-filtered, Hybri-Max<sup>®</sup> DMSO (Product Code D 2650).

#### Storage/Stability

Store the cocktail at  $-20^{\circ}\text{C}$ . The product, as supplied, is stable for 2 years when stored at  $-20^{\circ}\text{C}$ , 8 months at  $2-8^{\circ}\text{C}$ , and 2 months at room temperature.

#### Procedure

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 concentrations of DMSO greater than 0.5% may be deleterious to cell growth. Further dilutions, 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**, 405-411 (1984).
3. Aoyagi T., and Umezawa, H., Acta Biol. Med. Ger., **40**, 1523-1529 (1981).
4. Mumford, R. A. et al, Biochem. Biophys. Res. Commun., **103**, 565-572 (1981).

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