

# **Product Information**

# ReadyShield<sup>™</sup> Protease and Phosphatase Inhibitor Cocktail

Cat. No. PPC2020

Storage Temperature -20 °C

## **Product Description**

Crude protein extracts contain endogenous enzymes, such as proteases and phosphatases, which can degrade or dephosphorylate the proteins in the extracts. The best way to increase the yield of intact unmodified proteins is to add inhibitors for the protease and phosphatase enzymes known to be present in the extract.

ReadyShield™ Protease and Phosphatase Inhibitor Cocktail has been optimized and tested for mammalian cellular and tissue extracts. It contains inhibitors with a broad specificity for serine, cysteine, acid proteases, aminopeptidases, L-isozymes of alkaline phosphatases, and serine/ threonine protein phosphatases.

ReadyShield™ Protease and Phosphatase Inhibitor Cocktail has been tested on extracts from various animal tissues such as human placenta and bovine liver, and on extracts of A431, CHO, and U937 cells.

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

ReadyShield™ Protease and Phosphatase Inhibitor Cocktail is a non-freezing formulation that contains the same inhibitors as the Protease and Phosphatase Inhibitor Cocktail (Cat. No. PP1010).

Specific inhibitory properties of the components are:

- AEBSF [4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride] – serine proteases, e.g., trypsin, chymotrypsin, plasmin, kallikrein, and thrombin.
- Aprotinin serine proteases, e.g., trypsin, chymotrypsin, plasmin, and kallikrein; human leukocyte elastase, but not pancreatic elastase.
- Bestatin hydrochloride-aminopeptidases, e.g., leucine aminopeptidase and alanyl aminopeptidase.<sup>1-4</sup>
- E-64 [N-(trans-Epoxysuccinyl)-Lleucine 4-guanidinobutylamide] – cysteine proteases, e.g., calpain, papain, cathepsin B, and cathepsin L.
- Leupeptin hemisulfate salt– both serine and cysteine proteases, e.g., plasmin, trypsin, papain, and cathepsin B.
- Pepstatin A acid proteases, e.g., pepsin, renin and cathepsin D, and many microbial aspartic proteases.<sup>5</sup>
- Cantharidin (Cat. No. C7632 or equivalent) inhibits protein phosphatase 2A (PP-2A).
- (-)-p-Bromolevamisole oxalate (Cat. No. 190047 or equivalent) inhibits L-isoforms of alkaline phosphatases.<sup>6</sup>
- Calyculin A (Cat. No. C5552 or equivalent) inhibits protein phosphatases 1 and 2A (PP-1 and PP-2A).<sup>7,8</sup>



## **Precautions and Disclaimer**

This product is 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 cocktail is shipped on wet ice and storage at -20 °C is recommended. The product, as supplied, is stable for two years.

#### **Procedure**

The recommended dilution of the cocktail in the biological extract is 1 ml of the cocktail per 500 mg of protein extracted from tissue or cells. In many cases, the cocktail can be used at a final concentration of 1% (v/v), 1 ml of cocktail solution per 100 ml of extraction buffer.

**Note**: Not all lysates and extracts contain the same levels of endogenous enzymes, and it may be necessary to adjust the volume of cocktail used.

#### 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. Comm., **103**, 565-572 (1981).
- Honkanen, R.E., Cantharidin, another natural toxin that inhibits the activity of serine/threonine protein phosphatase types 1 and 2A. FEBS Lett., 330, 283-286 (1993).
- 6. Onsgard-Meyer, M. et al., Effect of bromotetramisole on renal phosphate excretion. Proc. Soc. Exp. Biol. Med., **213**, 193-195 (1996).

- 7. Runnegar, M.T. et al., Differential toxicity of the protein phosphatase inhibitors microcystin and calyculin A. J. Pharmacol. Exp. Ther., **273**, 545-553 (1995).
- Peirce, M.J. et al., Role of protein phosphatase in the regulation of human mast cell and basophil function.
  Am. J. Physiol., 277, C1021-C1028 (1999)

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