Phosphatase Inhibitor Cocktail 3
For Serine/Threonine Protein Phosphatases and L-Isozymes of Alkaline Phosphatase

Catalog Number P0044
Storage Temperature 2–8 °C

Product Description
Crude cell extracts contain a number of endogenous enzymes, such as proteases and phosphatases, which are capable of modifying the proteins present in the extract. The best way to improve the yield of intact proteins is to add inhibitors to these enzymes, which may be present in the source material.

Phosphatase Inhibitor Cocktail 3 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. It has been optimized and tested for L-isozymes of alkaline phosphatase as measured with p-nitrophenyl phosphate (pNPP) at pH 10.4, as well as for serine/threonine protein phosphatases (protein phosphatases 1 and 2A) as measured by dephosphorylation of 32P-Ser phosphorylase a at pH 7.5.

Components
The cocktail is supplied as a clear solution in dimethyl sulfoxide (DMSO). The individual components of this proprietary formulation have specific inhibitory properties:

Cantharidin (Catalog Number C7632 or equivalent) inhibits protein phosphatase 2A (PP-2A).

(−)-p-Bromolevamisole oxalate (Catalog Number 190047 or equivalent) inhibits L-isoforms of alkaline phosphatases.

Calyculin A (Catalog Number C5552 or equivalent) inhibits protein phosphatases 1 and 2A (PP-1 and PP-2A).

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 2–8 °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).

A 1% (v/v) final concentration of the cocktail in extraction buffer will inhibit phosphatase activities found in the 27,000 × g supernatant from human placenta and bovine liver, and in the 100,000 × g supernatant from A431, CHO, and U937 cell extracts, with a protein concentration of ~5 mg/ml.

References