PROMETHEUS™
Protein Biology Products

Instruction Manual

Protease Inhibitor Cocktail, Mammalian [100X] Cat. Nos.: 18-427, 18-428 & 18-429

Introduction:

The mammalian cell and tissue extracts contain a number of endogenous proteases, which are capable of modifying the proteins present in the extract. The need of protease inhibitors arises to protect the proteins from damage caused by the proteases. Moreover, to improve the yield of native proteins, use of protease inhibitor cocktail along with phosphatase and other inhibitors is recommended during the extraction process.

Our Protease Inhibitor Cocktail, Mammalian [100X] contains optimized concentration of protease inhibitors: AEBSF (4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride), Aprotinin, Bestatin, E-64 (N-[trans-Epoxysuccinyl]-L-leucine 4-guanidinobutylamide), Leupeptin and Pepstatin A protease inhibitors and other proprietary component(s) for broad spectrum inhibition of proteases that target serine proteases (e.g., trypsin, chymotrypsin, plasmin, kallikrein, thrombin, and human leukocyte elastase, but not pancreatic elastase), cysteine proteases (e.g., calpain, papain, cathepsin B, and cathepsin L), aminopeptidases (e.g., leucine aminopeptidase and alanyl aminopeptidase), acid proteases (e.g., pepsin, renin and cathepsin D), and many microbial aspartic proteases. It is supplied as a ready to use solution at 100X concentration, which makes it easier to use in low volume at 1X, 2X or more for samples with high protease activities and is better or equivalent in performance when compared with Roche tablet and Sigma's protease inhibitor cocktails. This inhibitor cocktail helps in preserving the proteins from degradation by proteases and can be used during the protein extraction from different mammalian cells and tissues.

Items Supplied:

Catalog No.	Product Name	Size	Storage Condition*
18-427	Protease Inhibitor Cocktail, Mammalian [100X]	1.0 ml	4°C to -20°C
18-428	Protease Inhibitor Cocktail, Mammalian [100X]	2.0 ml	4°C to -20°C
18-429	Protease Inhibitor Cocktail, Mammalian [100X]	5.0 ml	4°C to -20°C

**The product is shipped at ambient temperature and upon receipt, store it at 4°C for short-term storage (up to 4 months) and at -20°C for long term storage (up to 1 year). The product is stable for up to one year, if stored and used as recommended.*

Product Handling / Safety Warning:

This product is for research use only and must not be used for clinical diagnostics, therapeutic and human purposes. Wear gloves and other protective measures when handling it and avoid contact to eyes, skin and other exposed parts of the body, read safety data sheet for further details.

Important NOTE About the Product:

- The Protease Inhibitor Cocktail DOES NOT contain Phosphatase inhibitors, but only broad-spectrum protease inhibitors.
- The Protease Inhibitor Cocktail is EDTA Free. EDTA inhibits metallo-proteases by chelating the divalent cations necessary for their activity. By this same mechanism, the activities of

other proteins of interest may be affected when EDTA is used. Therefore, empirical testing may be required to determine if use of EDTA is beneficial in particular experiments.

- EDTA must be removed from the buffered protein solution by de-salting or dialysis, if the protein of interest is to be purified using immobilized metal chelate affinity chromatography or analysis by 2-D gel electrophoresis.
- The protease inhibitor cocktail is supplied as a 100X stock, which is normally effective when used at 1X concentration. For Example: 1 ml of 100X inhibitor cocktail is good for the inhibition of proteases found in 10 ml of cell lysate obtained from CHO cells at a cell density of 10^8 cells per ml culture. However, not all lysates contain the same levels of proteases/activity, and it may be necessary to adjust the volume of protease inhibitor cocktail as per the sample.

Instructions to Use the Product:

I. Take out the Protease Inhibitor Cocktail, Mammalian [100X] vial from refrigerator/freezer and let it equilibrate/thaw to room temp. Vortex briefly to mix the cocktail solution, if crystals develop due to storage in cold condition, vortex it for briefly for another 2-3 times.
II. The cocktail is supplied at 100X concentration and recommended to use at 1-2X concentration. Some protein samples may contain high levels of proteases, and optimization of the working concentration of the inhibitor cocktail to 2-3X or higher may be required.
III. Use 10 µl of Protease Inhibitor Cocktail [100X] in 1 ml of lysis buffer or lysate to achieve 1X concentration of the cocktail and use higher concentration [2-3X] of inhibitor cocktail, if required.
IV. If the protein extraction buffer doesn't contain EDTA in it, 10 µl of 0.5M EDTA (not supplied) per ml sample can be added for the inhibition of metalloproteases at 1X concentration along with Protease Inhibitor Cocktail. Higher concentration of EDTA 20 µl to 30 µl of 0.5M EDTA per ml sample can be added, if needed. However, addition of EDTA can be eliminated and metalloprotease activity is not a concern.

RELATED PRODUCTS:

1. **Protein Extraction Buffers/ Kits (Cat. No. 18-400, 18-402, 18-404, 18-406, 18-409, 18-411)**
   For extracting proteins from Bacteria, Insects Cells, Mammalian cells, Tissues and Yeast samples
2. **RIPA Lysis Buffer (18-415, 18-416 and 18-417)**
   For extracting proteins from different species samples.
3. **Protein Loading Buffer [2X] (Cat. No. 20-309)**
   Non-reducing ready to use buffer for loading protein samples on to the gel. Premixed, just add an equal volume to your protein sample
4. **Protein Loading Buffer [2X] (Cat. No. 20-310)**
   Reducing ready to use buffer for loading protein samples on to the gel. Premixed, just add an equal volume to your protein sample

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