

**Protease Inhibitor Cocktail, Bacterial [100X]**

**Cat. No.: 18-424, 18-425 & 18-426**

**Introduction:**

The bacterial cell 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. Protease Inhibitor Cocktail, Bacterial [100X] provides complete protection of proteins from degradation by endogenous proteases that may be released during extraction and purification of proteins from animal and plant tissues, cultured cells, yeast or bacteria.

Our Protease Inhibitor Cocktail, Bacterial [100X] contains optimized concentration of protease inhibitors: AEBSF, E-64, Bestatin, and Pepstatin protease inhibitors and other proprietary component(s) for broad spectrum inhibition of proteases that target serine proteases (e.g. trypsin, chymotrypsin, plasmin, kallikrein, and thrombin), cysteine proteases (e.g. calpain, papain, cathepsin B, and cathepsin L), aminopeptidases (e.g. leucine aminopeptidase and alanyl aminopeptidase), and acid proteases (e.g. pepsin, rennin, and cathepsin D) and microbial aspartic proteases. It is supplied as a ready to use solution at 100X concentration, which makes it easier to use in low volumes at 1X, 2X or more for samples with high protease activities. This inhibitor cocktail helps in preserving the proteins from degradation by bacterial proteases and can be used during protein extracts from different species.

**Items Supplied:**

| Catalog No. | Product Name                                  | Size   | Storage*     |
|-------------|---|--------|--------------|
| 18-424      | Protease Inhibitor Cocktail, Bacterial [100X] | 1.0 ml | 4°C to -20°C |
| 18-425      | Protease Inhibitor Cocktail, Bacterial [100X] | 2.0 ml | 4°C to -20°C |
| 18-426      | Protease Inhibitor Cocktail, Bacterial [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 3 months) and at -20°C for long term storage (up to 1 year). When diluted to 1X in buffer, the protease inhibitors remain stable and active for 1-2 weeks at 4°C and up to 10-12 weeks at -20°C.*

**Product Handling / Safety Warning:**

This product is for research use only and must not be used for therapeutic or diagnostic purpose in human or animals. 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 enough for the inhibition of proteases found in 100 ml of bacterial lysate obtained from about 20 g wet pellet of *E. coli* cells or 10 g wet pellet of baculovirus-infected cells.

#### **Instructions to Use the Product:**

- I. Take out the Protease Inhibitor Cocktail, Bacterial [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 additional 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  $\mu$ l 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], if required.
- IV. If the protein extraction buffer doesn't contain EDTA in it, 10  $\mu$ l 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  $\mu$ l to 30  $\mu$ l of 0.5M EDTA per ml sample can be added, if needed.

#### **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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