

Instruction Manual

Protease Inhibitor Cocktail, His-Tag [100X]

Catalog No.: 18-433, 18-434 & 18-435

Introduction:

The host cell extracts for Histidine-Tagged (His-Tag) protein contain a number of endogenous proteases, which are capable of modifying the proteins present in the extract/lysate. The need of protease inhibitors arises to protect the proteins from damage caused by the proteases released during the lysis. Also, 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, His-Tag [100X] has a broad specificity for the inhibition of serine, cysteine, acid, thermolysin-like proteases, and aminopeptidases. It contains optimized concentration of protease inhibitors: AEBSF (4-[2-Aminoethyl] benzenesulfonyl fluoride hydrochloride), Bestatin, Pepstatin A, E-64 (N-[trans-Epoxysuccinyl]-L-leucine 4-guanidinobutylamide), Phosphoramidon 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, acid proteases (e.g., pepsin, rennin, and cathepsin D, and many microbial aspartic proteases), and membrane metallo-endopeptidase (e.g., thermolysin and collagenase), etc. The inhibitor cocktail does not contain any chelators, such as EDTA, to optimize its use in the purification of histidine-tagged proteins.

The inhibitor cocktail is supplied as a ready to use solution in DMSO, at 100X concentration, which makes it easier to use in low volume at 1X, 2X or more for samples with high protease activities. The supplied inhibitor cocktail helps in preserving the proteins from degradation by proteases and can be used with the lysis buffer for His-Tag protein extractions or lysate.

Catalog No.	Product Name	Size	Storage Condition*
18-433	Protease Inhibitor Cocktail, His-Tag [100X]	1.0 ml	4°C to -20°C
18-434	Protease Inhibitor Cocktail, His-Tag [100X]	2.0 ml	4°C to -20°C
18-435	Protease Inhibitor Cocktail, His-Tag [100X]	5.0 ml	4°C to -20°C

Items Supplied:

*The product is shipped at ambient temperature and upon receipt, store it at 4°C for shortterm storage (up to 4 months) and at -20°C for long term storage (up to 1 year). The product is stable for 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 or animal 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 for Histidine-Tagged protein extraction.
- The protease inhibitor cocktail is supplied as a 100X stock, which is normally effective when used at 1X concentration. For Example: 1 ml of cocktail solution is recommended for the inhibition of endogenous proteases found in 100 ml of lysate from ~ 20 g wet pellet of *E. coli* or ~ 10 g wet pellet of baculovirus-infected cells.

Instructions to Use the Product:

- Take out the Protease Inhibitor Cocktail vial from refrigerator/freezer and let it equilibrate/thaw to room temp. Vortex briefly to mix the 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 1X as a good starting point. However, the working concentration should be optimized for each individual application and 2-3X concentration can be used for samples with high protease activities.
- III. Add 10 μ l of Protease Inhibitor Cocktail, His-Tag [100X] directly into 1 ml of lysis buffer or lysate to achieve 1X concentration of the cocktail and use higher concentration [2-3X], if required.

RELATED PRODUCTS:

- 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. <u>RIPA Lysis Buffer (18-415, 18-416 and 18-417)</u> For extracting proteins from different species samples.
- 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. <u>Protein Loading Buffer [2X] (Cat. No. 20-310)</u> 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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