

Broad Spectrum Protease Inhibitor Cocktail (100x)

Cat#: orb76032 (Protocol)

List of Components

Description	Quantity	Volume	Contents
Broad Spectrum Protease Inhibitor Cocktail	1	1mL(100X)	Containing AEBSF, aprotinin, bestatin, E-64, leupeptin and pepstatin A stabilized in dimethylsulfoxide (DMSO)
0.1M EDTA solution	1	1mL(100X)	EDTA solution for optional metalloprotease inhibition

Overview

Product Name	Broad Spectrum Protease Inhibitor Cocktail
Physical State	Liquid, colorless, transparent
Pack Size	1mLX2
Safety Precautions	Harmful. DMSO is toxic and causes irritation to the eyes and skin. Please operate with caution and wear eye and hand protection and proper lab garments.
Recommended working concentration	100-fold dilution in lysis buffer 10 µL of the Protease Inhibitor Cocktail solution is enough to inhibit degradation of proteins in 1 mL lysate.
Storage	Upon receipt store at -20°C. It is stable for one year. Product is shipped on ice.
Equivalent	Thermofisher (Product No.78430, A32963)

Notes:

Type of DAPI	Content
Broad Spectrum Protease Inhibitor Cocktail	Broad Spectrum Protease Inhibitor Cocktail, 1mL (100X) 0.1M EDTA solution, 1mL (100X)
Broad Spectrum Protease Inhibitor Cocktail (EDTA free)	Broad Spectrum Protease Inhibitor Cocktail, 1mL (100X)

Biochemical Information

Protease Inhibitor Component	MW	Protease Family Targeted	Inhibition Type	Typical Working (1X) Conc.
E-64	357.4	Cysteine proteases (papain, calpain, lysosomal cathepsins)	Irreversible	15µM

AEBSF	239.5	Serine proteases (trypsin, chymotrypsin, plasmin, trypsinogen, urokinase, kallikrein)	Irreversible	1mM
Bestatin	308.4	Amino-peptidases	Reversible	50µM
Leupeptin	475.6	Serine and cysteine proteases	Reversible	20µM
Aprotinin	6511.5	Serine proteases (trypsin, chymotrypsin, plasmin, trypsinogen, urokinase, kallikrein)	Reversible	800nM
Pepstatin A	685.9	Aspartic acid proteases (pepsin and rennin)	Reversible	10µM
EDTA	372.2	Metalloproteases (thermolysin and carboxypeptidase A)	Reversible	1mM

Assay Principle

Broad Spectrum Protease Inhibitor Cocktail (100X) is a Western blot related ready-to-use concentrated stock solution reagent containing a blend of seven protease inhibitors that is to be added to cell lysis buffer to protect the integrity and functionality of native cellular proteins against degradation by multiple classes of endogenous proteases during protein extraction and sample preparation procedures. The product is supplied as a 100X concentrated stock solution in a liquid format for improved accuracy, solubility, and ease of use in comparison to traditional tablets.

Properties

Compatibility with reagents	Fully compatible with cell lysis buffers and Broad Spectrum Phosphatase Inhibitor Cocktail
Compatibility with assays	Not MS-compatible: contain AEBSF; Not compatible with immobilized metal chelate affinity chromatography and 2D gel electrophoresis: contain EDTA
Reagent Type	Western Blotting related reagent; Inhibitors
Usage	Protect native cellular proteins from destructive degradation by endogenous proteases following cell lysis Preserve native cellular proteins intact and functional Screen extracts for proteolytic activity Study proteolysis in the regulation of cellular processes
Target Specificity	Serine proteases, cysteine proteases, aspartic acid proteases, metalloproteases, aminopeptidases
Target Sample	Cell lysis extracts
Description	Biorbyt's Broad Spectrum Protease Inhibitor Cocktail is a complex of various protease inhibitors, which has been tested for inhibiting proteases and esterase broadly.
Cite This Product	Broad Spectrum Protease Inhibitor Cocktail.
Application	Western blotting, protein purification, Co-IP; assays for protein expression, activity, modification, profiling and characterization, quantitative

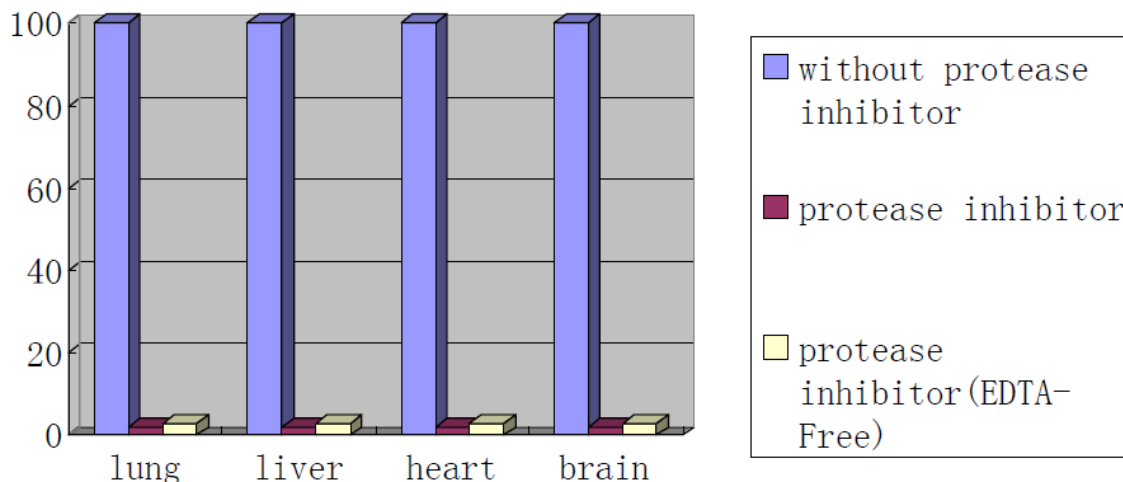
measurement; epitope tagging; reporter gene analysis

Background

Crude cell extracts contain a number of endogenous enzymes, such as proteases and phosphatases, which are capable of digesting the proteins present in the extract. An optimized method to improve the yield of intact and functional native proteins is to add inhibitors of these enzymes known to be present in the source material. This broad spectrum protease inhibitor cocktail is a complex of various protease inhibitors, which has been tested for inhibiting proteases and esterase broadly.

Protease inhibitors are critical reagents for the preservation of protein integrity during purification and analysis procedures by knocking out specific proteases to avoid peptide bond hydrolysis and subsequent protein destruction. They are biological or chemical compounds that function by reversibly or irreversibly binding to the protease. Most known proteases belong to one of four evolutionarily distinct enzyme families based on the functional groups involved in the peptide bond cleavage. No single chemical is effective for all types of proteases. A mixture or "cocktail" of several different inhibitor compounds is commonly used that consistently inhibit a multitude of protease classes to ensure that protein extracts do not degrade before analysis for target proteins of interest. Not using protease inhibitors leads to the loss of a large number of valuable proteins in a lysate sample, adversely affecting downstream applications by biologically meaningless representation of protein activities and gaining false negative immunostaining results for targets of interest.

Result image



Protease activity for extracted protein from mouse tissue while using different protease inhibitor cocktails