

CERTIFICATE OF ANALYSIS

**ProteoBlock™ Protease
Inhibitor Cocktail**

#R1321

Lot: Expiry Date:

Components

ProteoBlock™	
Protease Inhibitor Cocktail (100X)	2 x 1.25 ml
EDTA, 0.5 M	3 x 1 ml

Store at -20°C

Introduction

Proteases are proteolytic enzymes that are present in all living cells and rapidly degrade proteins during extraction procedures. The resulting proteolytic damage significantly decreases the quality and yields of sample proteins. Therefore, addition of a protease inhibitor cocktail to the extraction buffer is highly recommended to protect proteins from degradation.

Description

The ProteoBlock™ Protease Inhibitor Cocktail is a mixture of 6 different protease inhibitors ensuring inhibition of a broad range of proteases and esterases (*see* Table 1). The protease inhibitor cocktail is supplied as a ready-to-use solution at a 100X concentration in DMSO. EDTA is provided in a separate vial. Each pack of ProteoBlock™ Protease Inhibitor Cocktail supplied is sufficient to prepare 250 ml of extraction buffer.

Table 1. Components of the ProteoBlock™ Protease Inhibitor Cocktail.

Component	Concentration (100X)	Target proteases
AEBSF HCl	100 mM	Serine proteases
Aprotinin	80 µM	Serine proteases and esterases
Bestatin	5 mM	Aminopeptidases
E64	1.5 mM	Cysteine and thiol proteases
Leupeptin	2 mM	Cysteine and thiol proteases
Pepstatin A	1 mM	Acid proteases, many aspartic proteases
EDTA	0.5 M	Metalloproteases

Important Notes

- EDTA, an inhibitor of metalloproteases, is provided in a separate tube along with the ProteoBlock™ Protease Inhibitor Cocktail.
- The presence of EDTA in the extraction buffer is not compatible with some downstream applications. EDTA interferes with immobilized metal-chelate affinity chromatography (IMAC) and 2-D gel electrophoresis. The activity of some proteins can also be affected by EDTA.
- If required, EDTA should be added directly into the extraction buffer.

- Some sample types where protease levels are extremely abundant may require a higher concentration of the protease inhibitor. Therefore, optimization of inhibitor cocktail concentration may be required.
- Before each use, warm the cocktail solution at room temperature until it becomes the liquid.

PROCEDURE

Note. Wear gloves when working with the DMSO-containing protease inhibitor cocktail.

1. Warm the ProteoBlock™ Protease Inhibitor Cocktail to room temperature.
2. Vortex the tube to ensure a homogeneous suspension.
3. Just before use, add 10 µl of ProteoBlock™ Protease Inhibitor Cocktail per ml of extraction buffer to produce a 1X final concentration.
4. Optional: For inhibition of metalloproteases, add 10 µl of 0.5 M EDTA per 1 ml of extraction buffer to produce a final concentration of 5 mM.

(continued on reverse page)

Troubleshooting

Problem	Possible Cause and Solution
Inefficient inhibition of protease activity	1. Sample contains high levels of proteases <ul style="list-style-type: none">- Increase the final concentration of inhibitor cocktail to 2X or 3X.- If metalloproteases are present in the lysates, add EDTA to the extraction buffer. 2. Protein sample contains classes of proteases not inhibited by the cocktail <ul style="list-style-type: none">- Supplement the extraction buffer with individual inhibitors for the specific protease.

QUALITY CONTROL

The ProteoBlock™ Protease Inhibitor Cocktail is functionally tested in a validated spectrophotometric protease assay using pancreas extract.

Quality authorized by:

 Jurgita Zilinskiene

Related Products

- ProteoJET™ Mammalian Cell Lysis Reagent #K0301
- ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit #K0311
- ProteoJET™ Membrane Protein Extraction Kit #K0321
- DualColor™ Protein Loading Buffer Pack #R1011
- Spectra™ Multicolor Broad Range Protein Ladder #SM1841
- Spectra™ Multicolor High Range Protein Ladder #SM1851
- Spectra™ Multicolor Low Range Protein Ladder #SM1861
- Bradford Reagent, ready-to-use #R1271
- Bovine Serum Albumin Standard Set, ready-to-use #R1281
- Bovine Gamma Globulin Standard Set, ready-to-use #R1291
- PageBlue™ Protein Staining Solution #R0571
- PageSilver™ Silver Staining Kit #K0681

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.fermentas.com for Material Safety Data Sheet of the product.