

HaltTM Protease and Phosphatase Inhibitor Single-Use Cocktail

78442

Number Description

Halt Protease and Phosphatase Inhibitor Single-Use Cocktail, sufficient to treat 240mL of sample

Contents:

Inhibitor Single-Use Cocktail (100X), $100\mu L \times 24$ microtubes

EDTA Solution, 0.5M (100X), 2.5mL

Storage: Upon receipt store at 4°C. Product shipped with an ice pack. Do not freeze product.

Introduction

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The Thermo Scientific Halt Protease and Phosphatase Inhibitor Single-Use Cocktail prevents enzymatic protein degradation during extraction and purification procedures. The ready-to-use cocktail contains a mixture of several potent inhibitors (Table 1) against the major classes of endogenous proteases and phosphatases. The protease inhibitors target aminopeptidases, cysteine and serine proteases, and, when EDTA is included, metalloproteases. The phosphatase inhibitors target serine/threonine and protein tyrosine phosphatases. The refrigerated, single-use format maintains inhibitor potency by eliminating freeze-thaw cycles.

Table 1. Protease and phosphatase inhibitors included in the cocktail.

Inhibitor	<u>Target</u>
Aprotinin	Serine Proteases
Bestatin	Aminopeptidase Proteases
E-64	Cysteine Proteases
Leupeptin	Serine and Cysteine Proteases
Sodium Fluoride	Serine and Threonine Phosphatases
Sodium Orthovanadate	Tyrosine Phosphatases
Sodium Pyrophosphate	Serine and Threonine Phosphatases
β-glycerophosphate	Serine and Threonine Phosphatases
EDTA*	Metalloproteases

^{*}Provided in a separate vial.

Important Product Information

- This inhibitor cocktail is generally effective when used at a 1X final concentration; however, samples that contain high levels of proteases or phosphatases might require a more concentrated treatment (i.e., 2-3X).
- EDTA inhibits metalloproteases by chelating divalent cations necessary for their activity. By this same mechanism, EDTA might affect the activities of other proteins. Empirically determine if EDTA is beneficial for each experiment.
- This inhibitor cocktail interferes with immobilized metal-chelate affinity chromatography (IMAC) and 2D gel electrophoresis. Either dialyze or desalt (see Related Thermo Scientific Products Section) sample to effectively remove inhibitors from sample extracts before performing such procedures.
- Pepstatin A is not included in the cocktail formulation. If you need to inhibit acid proteases, purchase Pepstatin A (see Related Thermo Scientific Products) and add it to the cocktail.



Procedure

- 1. Remove the block of cocktail from the foil pouch. Use scissors to carefully remove one tube from the block. Return unused tubes to the foil pouch and place at 4°C immediately after removing the required tubes.
 - **Note:** To preserve the integrity of the foil seals, use scissors to cut the block. Do not tear the tubes from the block, which will compromise the seals of neighboring tubes.
- 2. Vortex the tube before use to ensure a homogeneous suspension. Puncture the foil seal with a pipette tip.
- 3. Add the single-use cocktail at 10µL/mL directly to the lysis buffer or extract to produce a 1X final concentration.
- 4. Optional: For inhibition of metalloproteases, add EDTA at 10μL/mL to the lysis buffer or extract to achieve a 1X (5mM) final working concentration.

Troubleshooting

Problem	Possible Cause	Solution
Poor inhibition of protease or phosphatase activity	Sample contains high levels of proteases or phosphatases	Add sufficient cocktail to produce a 2X or 3X final concentration
Cocktail does not provide sufficient protection against a particular protease family	Inhibitor for a specific family is not a component of the cocktail	Add individual inhibitors to the samples for the specific protease
	A specific inhibitor is not at the needed concentration	Use the cocktail at a higher concentration

Related Thermo Scientific Products

78445	Halt Protease and Phosphatase Inhibitor Single-Use Cocktail (100X), EDTA-free, $100\mu L \times 24$ microtubes
78441	Halt Protease and Phosphatase Inhibitor Cocktail EDTA-free (100X), 1mL
78445	Halt Protease and Phosphatase Inhibitor Cocktail EDTA-free (100X), $5 \times 1 \text{ mL}$
88663	Pierce® Protease and Phosphatase Inhibitor Tablets, 20 tablets
88664	Pierce Protease and Phosphatase Inhibitor Tablets, EDTA-free, 20 tablets
78428	Halt Phosphatase Inhibitor Single-Use Cocktail (100X), $100\mu L \times 24$ microtubes
88662	Pierce Phosphatase Inhibitor Tablets, 20 tablets
87785	Halt Protease Inhibitor Cocktail, EDTA-free (100X), 1mL
78437	Halt Protease Inhibitor Cocktail, EDTA-free (100X), 5mL
78439	Halt Protease Inhibitor Cocktail, EDTA-free (100X), 10mL
88660	Pierce Protease Inhibitor Tablets, 30 tablets
88661	Pierce Protease Inhibitor Tablets, EDTA-free, 30 tablets

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