INSTRUCTIONS

Halt™ Protease and Phosphatase Inhibitor Cocktail

78440 78444 78446

2053.2

Number	Description
78440	**Halt Protease and Phosphatase Inhibitor Cocktail**, sufficient for 100mL of sample **Contents:** **Protease and Phosphatase Inhibitor Cocktail** (100X), 1mL **EDTA Solution,** 0.5M (100X), 1mL
78444	**Halt Protease and Phosphatase Inhibitor Cocktail,** sufficient for 500mL of sample **Contents:** **Protease and Phosphatase Inhibitor Cocktail** (100X), 5 × 1mL **EDTA Solution,** 0.5M (100X), 5 × 1mL
78446	**Halt Protease and Phosphatase Inhibitor Cocktail**, sufficient for 1L of sample **Contents:** **Protease and Phosphatase Inhibitor Cocktail** (100X), 10mL **EDTA Solution,** 0.5M (100X), 10mL

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

Introduction

The Thermo Scientific Halt Protease and Phosphatase Inhibitor Cocktail protects proteins from degradation by endogenous proteases and phosphatases released during protein extraction and purification. The ready-to-use cocktail contains a mixture of several potent inhibitors (Table 1). The protease inhibitors target aminopeptidases, cysteine and serine proteases. The phosphatase inhibitors target serine/threonine and protein tyrosine phosphatases.

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

Inhibitor	Target Protease/Phosphatase
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.

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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 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 and add it to the cocktail.

Procedure

1. Vortex the bottle before use to ensure a homogeneous suspension.
2. Immediately before use, add the cocktail at 10µL/mL directly to the cell lysis buffer or extract to produce a 1X final concentration.
3. Optional: To inhibit metalloproteases, add EDTA at 10µL/mL of 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

78442 **Halt Protease and Phosphatase Inhibitor Single-Use Cocktail (100X),** 24 × 100µL

78441 **Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X),** 1mL

88663 **Pierce® Protease and Phosphatase Inhibitor Tablets,** 20 tablets

88664 **Pierce Protease and Phosphatase Inhibitor Tablets, EDTA-free,** 20 tablets

87786 **Halt Protease Inhibitor Cocktail,** 1mL

88660 **Pierce Protease Inhibitor Tablets,** 30 tablets

78420 **Halt Phosphatase Inhibitor Cocktail (100X),** 1mL

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