

# Halt Protease Inhibitor Single-Use Cocktail

78430

1987.4

Number	Description
78430	<b>Halt Protease Inhibitor Single-Use Cocktail</b> , sufficient to treat 240mL of sample
	<b>Contents:</b>
	<b>Protease Inhibitor Single-Use Cocktail (100X)</b> , 100 $\mu$ L $\times$ 24 microtubes
	<b>EDTA Solution, 0.5M (100X)</b> , 2.5mL

**Storage:** Upon receipt store product at 4°C. Product is shipped on ice pack.

## Introduction

The Thermo Scientific™ Halt™ Protease Inhibitor Single-Use Cocktail protects proteins from degradation by endogenous proteases that may be released during protein extraction and purification. The ready-to-use cocktail contains several potent inhibitors (see Table 1) in DMSO that inhibit the major classes of proteases including aspartic acid, cysteine and serine proteases, and when EDTA is included, metalloproteases. The refrigerated, single-use format maintains the potency of inhibitors by eliminating multiple freeze-thaw cycles. Also, each microtube conveniently contains 100 $\mu$ L that can treat up to 10mL of lysate.

## Important Product Information

- Halt Protease Inhibitor Single-Use Cocktail is a broad-spectrum protease inhibitor and does not contain phosphatase inhibitors. The protease inhibitors used in the formulation at their 1X working concentrations are listed in Table 1.
- EDTA inhibits metalloproteases by chelating the divalent cations necessary for their activity. By this same mechanism, the activities of other proteins may be affected by EDTA. Therefore, empirical testing may be required to determine if EDTA is beneficial in particular experiments.
- If the protein of interest is to be purified using immobilized metal chelate affinity chromatography (IMAC) or analyzed by 2D gel electrophoresis, EDTA must be removed by extensive dialysis or desalting before purifying. Alternatively, use Thermo Scientific Halt Protease Inhibitor Single-Use Cocktail EDTA-Free (Product No. 78245).
- The protease inhibitor cocktail is supplied as a 100X stock in DMSO and is generally effective when used at a final concentration of 1X; however, some sample types contain particularly high levels of proteases and optimization of the cocktail's working concentration may be required.
- This cocktail is typically a liquid at 4°C but occasionally might be frozen. Frozen product does affect the function or performance of the cocktail. If frozen, equilibrate the tube to room temperature or hold the tube between thumb and forefinger until the cocktail becomes a liquid.

**Table 1. Protease inhibitors included in the Halt Cocktail.**

<u>Inhibitor</u>	<u>1X</u>
AEBSF	1mM
Aprotinin	800nM
Bestatin	50 $\mu$ M
E64	15 $\mu$ M
Leupeptin	20 $\mu$ M
Pepstatin A	10 $\mu$ M
EDTA*	5mM

\*Provided in separate vial.

## Procedure

**Note:** Wear proper personal protective equipment when working with this DMSO-containing protease inhibitor cocktail.

1. Remove one strip of cocktail from the foil pouch. Use scissors to carefully cut off one tube from the strip. Return unused tubes to 4°C immediately after removing the required tubes. Do not allow unused cocktail to equilibrate to room temperature.

**Note:** To preserve the integrity of the foil seals, use scissors to cut the strip. Do not tear the tubes from the strip as this will compromise the seals of neighboring tubes.

2. Equilibrate the tube to room temperature.
3. Vortex the tube before use to ensure a homogeneous suspension. Puncture the foil seal with a pipette tip.
4. Add Halt Protease Inhibitor Single-Use Cocktail at 10µL/mL directly to the lysis buffer or cell extract to produce a 1X final concentration.

**Note:** Up to 10mL of lysis buffer or cell extract can be treated with one tube of cocktail.

5. Optional: For inhibition of metalloproteases, add the EDTA Solution at 10µL/mL to achieve a 1X (5mM) final working concentration.

## Troubleshooting

Problem	Possible Cause	Solution
Poor inhibition of protease activity	Sample contains high levels of protease	Add sufficient cocktail to produce a 2X or 3X final concentration
	EDTA component not added and metalloproteases remain active	Repeat experiment adding EDTA component
Cocktail does not provide sufficient protection against a particular protease family	Inhibitor for that family is not a component of the cocktail, or the cocktail does not contain the needed concentration	Add individual inhibitor to the sample for the specific protease family

## Related Thermo Scientific Products

<b>78425</b>	<b>Halt Protease Inhibitor Single-Use Cocktail EDTA-Free</b> , 100µL × 24 microtubes
<b>87785</b>	<b>Halt Protease Inhibitor Cocktail, EDTA-Free (100X)</b> , 1mL
<b>87786</b>	<b>Halt Protease Inhibitor Cocktail (100X)</b> , 1mL
<b>88660</b>	<b>Pierce Protease Inhibitor Tablets</b> , 30 tablets
<b>88661</b>	<b>Pierce Protease Inhibitor Tablets, EDTA-free</b> , 30 tablets
<b>78440</b>	<b>Halt Protease and Phosphatase Inhibitor Cocktail (100X)</b> , 1mL
<b>78441</b>	<b>Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-Free (100X)</b> , 1mL
<b>88663</b>	<b>Pierce Protease and Phosphatase Inhibitor Tablets</b> , 20 tablets
<b>88664</b>	<b>Pierce Protease and Phosphatase Inhibitor Tablets, EDTA-free</b> , 20 tablets

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