

## ProteoGuard™ EDTA-Free Protease Inhibitor Cocktail Protocol PT5140-2

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### A. General Considerations

#### 1. Storage

Store at 4°C upon receipt.

#### 2. Stability

Stable for 1 year from date of receipt under proper storage conditions.

#### 3. Protease Inhibitor Cocktail Components

Clontech's ProteoGuard EDTA-Free Protease Inhibitor Cocktail is a mixture of protease inhibitors that has been optimized to protect proteins from being digested by endogenous proteases that can be released during protein extraction from cell lysates. See Table 1 for individual inhibitor concentrations.

- The cocktail is a 100X stock solution in DMSO. A 100 µl aliquot may be used to treat up to 10 ml of lysate at a 1X concentration.
- Optimization may be necessary, since some samples can contain higher levels of proteases and require a higher concentration of cocktail to inhibit proteolytic activity.
- It is possible for the cocktail to freeze at 4°C, but its functionality will not be affected. It can be liquefied by holding the tube for a few minutes.

**Table 1. Components of 1X ProteoGuard EDTA-Free Protease Inhibitor Cocktail**

Protease Inhibitor	Concentration
Aprotinin	0.8 µM
Bestatin	50 µM
Leupeptin	20 µM
Pepstatin A	10 µM
PMSF	1 mM

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## B. Procedure

**NOTE:** Proper personal protective equipment is necessary when working with the ProteoGuard EDTA-Free Protease Inhibitor Cocktail.

1. Thaw the tube containing the cocktail to room temperature.
2. Vortex the tube to obtain a homogeneous solution.
3. Add 10 µl of Protease Inhibitor Cocktail per ml of protein extract or lysis buffer prior to lysing cells to yield a 1X final concentration of inhibitors.
4. Extract proteins following your usual procedures.
5. If poor inhibition of protease activity is observed, then the sample may contain high levels of protease. Add more cocktail to yield a 2X or 3X final concentration.

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