

## ProBlock™ Gold Mammalian Protease Inhibitor Cocktail Specific for Mammalian Cells

### Introduction

*ProBlock™ Gold Mammalian protease inhibitor cocktail* is an easy-to-use, mammalian protease inhibitor cocktail specifically developed for sample preparation and purification from mammalian cells and tissues. *ProBlock™ Gold Mammalian protease inhibitor cocktail* contains optimized concentrations of both reversible and irreversible protease inhibitors to inhibit mammalian serine, cysteine, and metalloproteases. It also contains specific inhibitors for mammalian proteases, such as aspartic proteases, aminopeptidases and trypsin-like proteases.

Optional solutions of EDTA and 1,10-Phenanthroline are provided to inhibit metalloproteases. No EDTA is present in the *ProBlock™ Gold Mammalian cocktail*. Since some proteins require divalent cations like  $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mg}^{2+}$  or  $\text{Mn}^{2+}$  for their biological activity, the presence of EDTA or 1,10-Phenanthroline may be detrimental to the protein activity. In addition, EDTA and or 1,10-Phenanthroline may inhibit the purification of proteins using immobilized metal affinity chromatography (IMAC).

*ProBlock™ Gold Mammalian protease inhibitor cocktail* inhibits over 95% of protease activities at 1X concentration (pH 7-8) in extraction buffer.

### Items Included

- [ProBlock™ Gold Mammalian \[100X\] \(GoldBio Catalog #GB-331\)](#)
- 0.5M EDTA
- 1M 1,10-Phenanthroline

### Storage Conditions

Shipped at ambient temperature. Upon arrival, store contents at 4°C. If stored properly, this product is stable for 1 year.\*

### Method

1. Allow the *ProBlock™ Gold Mammalian protease inhibitor cocktail* solution to warm to room temperature. This solution is in suspension form. Vortex the vial before removing any solution.
2. Add *ProBlock™ Gold Mammalian protease inhibitor cocktail* 10  $\mu\text{l}/\text{ml}$  directly to an appropriate volume of extraction buffer or protein extract to 1X final concentration.

For more potent protease inhibition, add *ProBlock™ Gold Mammalian protease inhibitor cocktail* 20-30 µl/ml to give 2-3X final concentration.

*\* When ProBlock™ Gold Mammalian is added to the buffer or extract, it is stable for 1-2 weeks at 4°C and 4-6 weeks at -20°C.*

3. Mix solution thoroughly.

**Note: (OPTIONAL).** For inhibition of metalloproteases (if the buffer does not contain EDTA), add 0.5M EDTA (10 µl/ml) and 1M 1,10-Phenanthroline (10 µl/ml) directly to an appropriate volume of extraction buffer or extract to 1X final concentration (See Table 1).

**Table 1. Optional EDTA and 1,10-Phenanthroline additives for metalloproteinase inhibition.**

Optional Additive	Inhibition	Preparation	Working concentration
EDTA	Broad spectrum metalloproteinase inhibition	10 µl in 1 ml extraction buffer	10mM
1,10-Phenanthroline	Broad spectrum metalloproteinase inhibition	10 µl in 1 ml extraction buffer	10mM