# **Protocol**



TD-P Revision 2.1

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## **ProBlock™ Gold Bacterial**

**Protease Inhibitor Cocktail for Bacteria** 

#### Introduction

ProBlock™ Gold Bacterial is an easy-to-use, bacterial protease inhibitor cocktail specifically developed for sample preparation of bacterial proteins. ProBlock™ Gold Bacterial contains optimized concentrations of various bacterial protease inhibitors, which provide excellent inhibition of protease activities during purification from bacteria. The cocktail contains both reversible and irreversible protease inhibitors to inhibit serine, cysteine, and metalloproteases. It also contains specific inhibitors for bacterial proteases, such as aspartic proteases and aminopeptidases.

An optional solution of EDTA is provided to inhibit metalloproteases. No EDTA is present in the *ProBlock™ Gold Bacterial* cocktail. Since some proteins require divalent cations like Ca<sup>2+</sup>, Mg<sup>2+</sup> or Mn<sup>2+</sup> for their biological activity, the presence of EDTA may be detrimental to the protein activity. In addition, EDTA would inhibit the purification of proteins using immobilized metal affinity chromatography (IMAC).

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

#### Items Included

- ProBlock™ Gold Bacterial [100X] (GoldBio Catalog #GB-330)
- 0.5M EDTA

### **Storage Conditions**

Shipped at ambient temperature. Upon arrival, store it refrigerated at 4°C. If stored properly, it is stable for 2 years\*.

#### Method

- 1. Allow solution to warm to room temperature. The solution is in suspension form, vortex the vial before removing the solution.
- 2. Add *ProBlock™ Gold Bacterial* 10 µl/ml directly in an appropriate volume of extraction buffer or protein extract to 1X final concentration. For more potent protease inhibition, add *ProBlock™ Gold Bacterial* 20-30 µl/ml to give 2-3X final concentration.



Gold Biotechnology/ FM-000008 ProBlock™ Gold Bacterial TD-P Revision 2.1 TD-P Date: 4/8/2022

\* When ProBlock™ Gold Bacterial 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  $\mu$ l/ml directly in an appropriate volume of extraction buffer or extract to 1X final concentration.

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