



Protocol

TD-P Revision 2.1

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ProBlock™ Plus EDTA Protease Inhibitor Cocktail for Large Volume

Introduction

ProBlock™ -50, Plus EDTA and ProBlock™ -100, Plus EDTA are easy-to-use, general protease inhibitor cocktails. Due to the optimized concentration of the various inhibitors, the ProBlock™ shows excellent, broad-spectrum inhibition of protease activities and is therefore suitable for the protection of proteins extracted from animal cells/ tissues, plants, yeast and bacteria etc. ProBlock™ contains both irreversible and reversible protease inhibitors and inhibits serine, cysteine and metalloproteases. At 1X concentration (pH 7-8) in extraction buffer, ProBlock™ inhibits over 90% of protease activities.

A separate solution of EDTA is provided with ProBlock™ Plus EDTA for inhibiting metalloproteases. Since some proteins require divalent cations like Ca^{2+} , Mg^{2+} or Mn^{2+} for their biological activity, the presence of EDTA may be detrimental to the protein activity. Furthermore, if the protein of interest is purified using immobilized metal chelate affinity chromatography (IMAC), EDTA must be removed from the buffer before the chromatography.

ProBlock™ -50, Plus EDTA is designed for large volumes of extraction/lysis buffers and is supplied as 10 vials /kit (Cat# GB-334-10) or 20 vials/kit (Cat# GB-334-20), with each vial suitable for 50 ml.

ProBlock™ -100, Plus EDTA (Catalog # GB-336-100) is designed for large volumes of extraction/lysis buffers and is supplied as 10 vials/kit with each vial suitable for 100 ml.

Items Included

- ProBlock™ Protease Inhibitor Cocktail -50, Plus EDTA (Catalog # GB-334) or ProBlock™ Protease Inhibitor Cocktail -100, Plus EDTA (Catalog # GB-336)
- ProBlock™ Buffer
- 0.5M EDTA

Storage Conditions

Shipped at ambient temperature. Upon arrival, store it refrigerated at 4°C. If stored properly, these products should be stable for at least 2 years from the date of receipt. Reconstituted ProBlock™ in buffer is stable for up to 1 week at 4°C or 4-6 weeks at -20°C.

Method



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ProBlock™ Plus EDTA

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1. Allow ProBlock™ Buffer to warm to room temperature.
2. Add 0.5 ml ProBlock™ Buffer directly to a vial of ProBlock™. Vortex briefly until completely dissolved. Close the ProBlock™ Buffer cap tightly.

Note: When ProBlock™ is added to the buffer, it is stable for up to 1 week at 4°C or 4-6 weeks at -20°C.

3. For ProBlock™ -50, add 0.5 ml of reconstituted ProBlock™ directly to 50 ml extraction/lysis buffer and mix thoroughly. For ProBlock™ -100, add 0.5 ml of reconstituted ProBlock™ directly to 100 ml extraction/lysis buffer and mix thoroughly.

Note: For more potent protease inhibition, the amount of reconstituted ProBlock™ may be doubled.

4. Once ProBlock™ is added in the extraction/ lysis buffer, use it within 20-30 minutes.

Note: (OPTIONAL). If metalloproteases inhibition is needed, add 500 µl 0.5M EDTA solution per 50 ml or 1000 µl of 0.5M EDTA solution per 100

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