

Protease Inhibitor Cocktails

<u>Code</u>	<u>Description</u>	<u>Size</u>
PG-122-1ml	Protease Inhibitor Cocktail, General Use Supplied as lyophilized powder. <i>Each vial can be reconstituted in 1 ml deionized water to form a 100X solution.</i>	1.0 ml
PG-052-1ml	Protease Inhibitor Cocktail, Mammalian Supplied as lyophilized powder. <i>Each vial can be reconstituted in 1 ml deionized water to form a 100X solution.</i>	1.0 ml
PG-603-1ml	Protease Inhibitor Cocktail, Bacterial Supplied as lyophilized powder. <i>Each vial contains sufficient material to provide 1 ml of a 100X solution upon reconstitution.</i>	1.0 ml
PG-703-1ml	Protease Inhibitor Cocktail, Plant Supplied as lyophilized powder. <i>Each vial can be reconstituted in 1 ml dimethyl sulfoxide to form a 100X solution.</i>	1.0 ml

General Information:

Protease inhibitors are critical reagents for the preservation of protein integrity during purification and analysis procedures. Inhibitor cocktails are extensively used to provide broad spectrum protection during cell lysis and tissue dissolution. These cocktails target a range of different proteases including serine, cysteine, aspartic and metalloproteases as well as aminopeptidases.

Puregene offers four protease inhibitor cocktails designed to preserve the protein composition of samples in a broad range of organisms. A general use cocktail is available without EDTA, while specific formulations are available for mammalian, bacterial or plant lysates. All formulations are prepared from ultrapure reagents to ensure maximum inhibition of the specified proteases. Cocktails are provided as convenient, easy-to-use powders that can be reconstituted in the supplied vials.

Storage/Stability:

The lyophilized powder should be stored at -20°C where it is stable for up to 1 year. The reconstituted solution should be stored at -20°C where it is stable for up to 2 weeks. Repeated freezing and thawing cycles of the reconstituted solution is not recommended.

Application Disclaimer

*For Research Use Only.
Not for Therapeutic or Diagnostic Use.*

Protocols and Formulations

➔ **Note:** Reconstituted cocktails should be aliquoted into multiple tubes and stored at -20°C. Multiple freezing and thawing cycles are not recommended.

PG-122-1ML, General Use Protease Inhibitor Cocktail

Preparation of 100X Solution:

1. Add 1 ml deionized, distilled water and mix gently until powder is completely resuspended.
2. Aliquot into multiple tubes and store at -20°C.

<u>Name</u>	<u>MW</u>	<u>100X Concentration</u>	<u>Recommended Working Concentration</u>
AEBSF	239.5	50.00 mM	0.50 mM
Aprotinin	6512.0	30.00 uM	0.30 uM
Bestatin	308.4	1.00 mM	10.00 uM
E-64	357.4	1.00 mM	10.00 uM
Leupeptin	493.6	1.00 mM	10.00 uM

PG-603-1ML Bacterial Protease Inhibitor Cocktail

Preparation of 100X Solution

1. Add 1 ml DMSO to vial and vortex 1 minute.
2. Aliquot into multiple tubes and store at -20°C. (solution may be cloudy and precipitate may be present)

<u>Name</u>	<u>MW</u>	<u>100X Concentration</u>	<u>Recommended Working Concentration*</u>
AEBSF	239.5	110.00 mM	1.10 mM
Bestatin	308.4	9.35 mM	9.35 uM
E-64	357.4	1.10 mM	11.00 uM
Pepstatin	685.9	11.00 mM	110.00 uM
EDTA	372.2	465.00mM	4.65 mM

*1 ml of solution is sufficient to inhibit 20 ml of lysate from 4 g (wet weight) of *E. coli* cells.

PG-052-1ML, Mammalian Protease Inhibitor Cocktail:

Preparation of 100X Solution:

1. Add 1 ml deionized, distilled water and mix gently until powder is completely resuspended.
2. Aliquot into multiple tubes and store at -20°C.

<u>Name</u>	<u>MW</u>	<u>100X Concentration</u>	<u>Recommended Working Concentration</u>
AEBSF	239.5	120.00 mM	1.20 mM
Aprotinin	6512.0	46.00 uM	0.46 uM
Bestatin	308.4	1.36 mM	14.00 uM
E-64	357.4	1.23 mM	12.30 uM
Leupeptin	493.6	11.20 mM	112.00 uM
Pepstatin	685.9	116.00 uM	1.16 uM

PG-703-1ML, Plant Protease Inhibitor Cocktail:

Preparation of 100X Solution:

1. Add 1 ml dimethyl sulfoxide and mix gently until powder is completely resuspended.
2. Aliquot into multiple tubes and store at -20°C.

<u>Name</u>	<u>MW</u>	<u>100X Concentration</u>	<u>Recommended Working Concentration</u>
AEBSF	239.5	220.00 mM	2.20 mM
Bestatin	308.4	10.70 mM	107.00 uM
E-64	357.4	3.00 mM	30.00 uM
Leupeptin	493.6	2.00 mM	20.00 uM
Pepstatin	685.9	2.20 mM	22.00 uM
1,10-Phenanthroline	198.2	550.00 mM	5.50 mM

Inhibitor Specificity

Name	Inhibition Specificity
AEBSF	• Irreversible serine protease inhibitor · Chymotrypsin · Trypsin · Kallikrein · Plasmin · Thrombin. Non-toxic alternative for PMSF
Aprotinin	• Competitive, reversible serine protease inhibitor · Chymotrypsin · Trypsin · Kallikrein · Plasmin Does not inhibit Factor Xa or thrombin
Bestatin	• Competitive aminopeptidases inhibitor · Aminopeptidase B · Leucine aminopeptidase · Tripeptide amonopeptidase Does not inhibit carboxypeptidases
E-64	• Irreversible cysteine protease inhibitor · Papain · Calpain · Cathepsin B, H, L and S Effective inhibitor of collagenase
EDTA	• Reversible metalloprotease inhibitor – Chelates metal ions
Leupeptin	• Reversible cysteine and serine protease inhibitor · Trypsin · Plasmin · Papain · Kallikrein · Thrombin · Cathepsin A and B Effective inhibitor of collagenase
Pepstatin	• Aspartic acid protease inhibitor · Pepsin · Renin · Cathepsin D Insoluble in water
1,10-Phenanthroline	• Metalloprotease inhibitor – Chelates iron and other divalent cations