

Proteases & Phosphatases Inhibitors, Enzymes & Assays *Featuring Inhibitor Selection Guides*



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Protease Inhibitor Cocktails INTRODUCTION

Protease Inhibitor cocktails and specific inhibitors to proteases are important in the protection of proteins from proteolysis in such applications as protein extraction, purification, electrophoresis, storage, assays etc.

During isolation and characterization of the proteins, proteases are released following cell or tissue lysis and degrade protein samples, which can reduce the quality of the protein sample for further analysis. In order to prevent degradation of the proteins, protease inhibitor cocktail is added, which help preserve the nature of the protein.

G-Biosciences offers a large selection of protease inhibitor cocktails, protease assays and screening systems, phosphatase inhibitor cocktails, as well as specific proteases for use in protein sequencing and mass spectrometry.

G-Biosciences offers ProteaseArrest[™], which is a broad range of protease inhibitor cocktails with wide species specificity. ProteaseArrest[™] cocktails are used for inhibition of protease activity in protein preparations of mammalian, bacteria, plant, yeast and fungal lysates.

General protease inhibitors and a large selection of individual protease inhibitors are offered separately or as a protease inhibitor set in addition to the ProteaseArrest[™] inhibitor cocktails. For the identification of specific proteases and to screen for the presence of proteases, several protease assays and screening systems are available. For the protection of protein phosphatase groups, PhosphataseArrest[™] Phosphatase Inhibitor Cocktails are offered.

PROTEASE INHIBITORS

ProteaseArrest[™]

A broad range protease inhibitor cocktail with wide species specificity

ProteaseArrest[™] is a general protease inhibitor cocktail solution that is provided as a 100X concentrated, ready-to-use solution. The ProteaseArrest[™] 100X solution format is suitable for small, analytical sample applications, as >95% inhibition is achieved by adding 10µl ProteaseArrest[™] per ml sample. For samples with higher than normal protease levels, the volume of ProteaseArrest[™] added can be increased for greater inhibition levels.

The cocktail contains reversible and irreversible inhibitors of serine, cysteine, calpain and metallo-proteases.

An optional EDTA solution is provided for enhanced metalloprotease inhibition. It is not present in the actual ProteaseArrest[™] cocktail as it would inhibit the activity of proteins that require divalent cations (Ca²⁺, Mg²⁺ or Mn²⁺) for their biological activity. In addition, EDTA will inhibit the purification of proteins using immobilized metal affinity chromatography (IMAC), including 6X His tagged recombinant proteins.

Due to the optimized concentration of the various inhibitors, ProteaseArrest[™] shows excellent inhibition of protease activities and is therefore suitable for the protection of proteins during preparation of samples and protein purification from animal tissues, plants, yeast and bacteria.

ProteaseArrest[™] is also available as single use aliquots that are suitable for >95% protease inhibition in 10ml solutions. These *OneQuant*[™] ProteaseArrest[™] are provided for additional protease inhibitor cocktail convenience.

The ProteaseArrest[™] format allows delivery of optimized concentrations of protease inhibitor, for example 2X or higher concentrations can be added for tissues with higher than normal protease concentrations; a feature not possible with tablet format protease inhibitor cocktails.

In our study, a 1X concentration of ProteaseArrest[™] inhibits over 95% of protease activities (e.g. 0.5mg/ml mouse pancreas extract). The ProteaseArrest[™] protease inhibitor cocktail demonstrated greater inhibition levels compared to similar protease inhibitor cocktails, including tablet formats. In independent studies, researchers have found that ProteaseArrest[™] outperforms several leading manufacturer's protease inhibitor cocktails, including tablet formats, in the purification of plant proteins.

COMPOSITION

 668µM AEBSF, 0.3µM Aprotinin, 3µM Bestatin, 5.25µM Leupeptin, 1mM PMSF, 5mM EDTA (Optional) in DMSO

FEATURES

- · Broad spectrum protease inhibitor cocktail
- 100X concentrated, ready-to-use solution
- High inhibition levels: 1X ProteaseArrest[™] inhibits >95% of protease activities (i.e. 0.5 mg/ml mouse pancreas extract)

APPLICATIONS

- Inhibition of protease activity in protein preparations of mammalian, bacteria, plant, yeast and fungal lysates
- Protection of proteins from proteolysis in such applications as electrophoresis, purification, storage, assays, and other applications

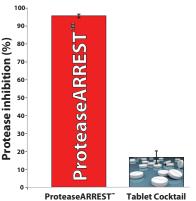


Figure 1: ProteaseArrest[™] outperforms tablet format protease inhibitor cocktails. Protease inhibition in mouse pancreas lysate with EDTAfree ProteaseArrest^m and a commercially available EDTAfree tablet protease cocktail were compared using Protease Screening[™] Kit. The assay used 0.5mg/ml pancreas lysate and incubation conditions of 37°C for 2.5 hours. ProteaseArrest™ inhibited over 95% of total proteases, 80% more compared to tablet inhibition.

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 - More references available at www.gbiosciences.com

Cat. No.	Description	Size
<u>786-108</u>	ProteaseArrest [™] [100X]	2ml
<u>786-437</u>	ProteaseArrest [™] [100X]	5ml
<u>786-711</u>	ProteaseArrest [™] [100X]	10ml
<u>786-712</u>	ProteaseArrest [™] [100X]	5 x 10ml
<u>786-329</u>	<u>OneQuant[™] ProteaseArrest[™] [100X]</u>	24 x 100µl

FOCUS[™] ProteaseArrest[™]

A 2D electrophoresis & mass spectrometry compatible protease inhibitor cocktail

A ready-to-use, 100X concentrated, broad range protease inhibitor cocktail that is fully compatible with 2D electrophoresis and subsequent mass spectrometry.

The protease inhibitor cocktail contains reversible and irreversible inhibitors of serine, cysteine, calpain and metallo- proteases. Due to the optimized concentration of the various inhibitors, the FOCUS[™] ProteaseArrest[™] shows excellent inhibition of protease activities and is therefore suitable for the protection of protein samples from animal tissues, plants, yeast and bacteria.

FOCUS[™] ProteaseArrest[™] is compatible with 2D electrophoresis as it uses an alternative to EDTA as an inhibitor of metalloproteases. The absence of EDTA allows for optimal action of nucleases for removing nucleic acids from the samples. In addition, FOCUS[™] ProteaseArrest[™] uses PMSF as its primary serine protease inhibitor as opposed to the commonly used Pefabloc®. Pefabloc® has been reported to modify proteins at high concentrations and result in artifacts in subsequent 2D electrophoresis and mass spectrometry.

COMPOSITION

 340µM AEBSF, 0.3µM Aprotinin, 1.45µM Bestatin, 3.4µM Calpain Inhibitor I, 5.25µM Leupeptin, 17µM Phosphoramidon, 1mM PMSF in DMSO

FEATURES

- 2D electrophoresis compatible, broad spectrum protease inhibitor cocktail
- 100X concentrated, ready-to-use solution
- High inhibition levels: 1X FOCUS[™] ProteaseArrest[™] inhibits >95% of protease activities (i.e. mouse pancreas extract, 0.5mg/ml protein)

APPLICATIONS

- · Inhibition of protease activity in protein preparations
- Suitable for 2D gel sample preparation for protection of protein samples

CITED REFERENCES

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 Wang, T. et al (2007) Biochem. Bioph. Res. Co. 352:203



Protease Inhibitor Cocktails

Plant ProteaseArrest[™]

A protease inhibitor cocktail enhanced with plant specific protease inhibitors

A plant, broad range, 100X concentrated, ready-to-use protease inhibitor cocktail. Plant ProteaseArrest[™] inhibits plant serine, cysteine and other plant specific proteases including aminopeptidases, aspartic and metalloproteases.

COMPOSITION

 668μM AEBSF, 3μM Bestatin, 14μM E-64, 5.25μM Leupeptin, 1μM Pepstatin A, 2mM 1,10-Phenathroline, 1mM PMSF in DMSO

FEATURES

- · Plant specific protease inhibitor cocktail
- 100X concentrated, ready-to-use solution

APPLICATIONS

- · Inhibition of proteases in protein preparations of plant lysates
- Protection of proteins from proteolysis in electrophoresis, purification, storage, assays, and other applications

CITED REFERENCES

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 - Krishnan, H. B. et al (2015) J. Agric. Food Chem. 63:2862

Cat. No.	Description	Size
<u>786-332</u>	Plant ProteaseArrest [™] [100X]	1ml
<u>786-434</u>	Plant ProteaseArrest [™] [100X]	5ml

Bacterial ProteaseArrest[™]

A protease inhibitor cocktail enhanced with bacterial specific protease inhibitors

A bacterial, broad range, 100X concentrated, ready-to-use protease inhibitor cocktail. Bacterial ProteaseArrest[™] inhibits bacterial serine, cysteine and other bacterial specific proteases including aminopeptidases and aspartic proteases.

An optional EDTA solution is provided for enhanced metalloprotease inhibition. It is not present in the actual Bacterial ProteaseArrest[™] cocktail as it would inhibit the activity of proteins that require divalent cations (Ca²⁺, Mg²⁺ or Mn²⁺) for their biological activity. In addition, EDTA will inhibit the purification of proteins using immobilized metal affinity chromatography (IMAC).

COMPOSITION

• 668 μ M AEBSF, 3 μ M Bestatin, 14 μ M E-64, 5mM EDTA (Optional), 1 μ M Pepstatin A, 1mM PMSF in DMSO

FEATURES

- · Bacteria specific protease inhibitor cocktail
- · 100X concentrated, ready-to-use solution

APPLICATIONS

- Inhibition of protease activity in protein preparations of bacterial lysates
- Protection of proteins from proteolysis in such applications as electrophoresis, purification, storage, assays, and other applications

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- 2. Wu, T. et al (2010) J Immunol 184:2183
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 Person, M.D. et al (2006) J Biomol Tech 17:145
 - Cat. No.DescriptionSize786-330Bacterial ProteaseArrest[™] [100X]1ml786-432Bacterial ProteaseArrest[™] [100X]5ml

Protease Inhibitor Cocktails

Mammalian ProteaseArrest™

A protease inhibitor cocktail enhanced with mammalian specific protease inhibitors

A mammalian, broad range, 100X concentrated, ready-to-use protease inhibitor cocktail. Mammalian ProteaseArrest[™] inhibits mammalian serine, cysteine and other mammalian specific proteases including aminopeptidases, trypsin-like and aspartic proteases. An optional EDTA solution is provided for enhanced metalloprotease inhibition. It is not present in the actual Mammalian ProteaseArrest[™] cocktail as it would inhibit the activity of proteins that require divalent cations (Ca²⁺, Mg²⁺ or Mn²⁺) for their biological activity. In addition, EDTA will inhibit the purification of proteins using immobilized metal affinity chromatography.

COMPOSITION

 668μm AEBSF, 0.3μM Aprotinin, 3μM Bestatin, 14μM E-64, 5mM EDTA (Optional), 5.25μM Leupeptin, 1μM Pepstatin A, 1mM PMSF in DMSO

FEATURES

- · Mammalian specific protease inhibitor cocktail
- 100X concentrated, ready-to-use solution

APPLICATIONS

- Inhibition of protease activity in protein preparations of mammalian lysates
- Protection of proteins from proteolysis in such applications as electrophoresis, purification, storage, assays, and other applications

CITED REFERENCES

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- More refernces available at www.gbiosciences.com

Cat. No.	Description	Size
<u>786-331</u>	Mammalian ProteaseArrest [™] [100X]	1ml
<u>786-433</u>	Mammalian ProteaseArrest [™] [100X]	5ml

Yeast/ Fungal ProteaseArrest™

A protease inhibitor cocktail enhanced with yeast and fungal specific protease inhibitors

A yeast and fungal, broad range, 100X concentrated, ready-to-use protease inhibitor cocktail. Yeast/ Fungal ProteaseArrest[™] inhibits yeast and fungal serine, cysteine and metalloproteases.

COMPOSITION

 668µM AEBSF, 14µM E-64, 1µM Pepstatin A, 2mM 1,10-Phenathroline, 1mM PMSF in DMSO

FEATURES

- · Yeast and fungal specific protease inhibitor cocktail
- 100X concentrated, ready-to-use solution

APPLICATIONS

- Inhibition of protease activity in protein preparations of yeast and fungal lysates
- Protection of proteins from proteolysis in such applications as electrophoresis, purification, storage, assays, and other applications

CITED REFERENCES

Rustagi, A. et al (2014) Molecular Biotechnology. 56:535
 Walliwalagedara, C. et al (2010) Open Proteomics. 3:20

Cat. No.	Description	Size
<u>786-333</u>	Yeast/ Fungal ProteaseArrest [™] [100X]	1ml
<u>786-435</u>	Yeast/ Fungal ProteaseArrest [™] [100X]	5ml

Broad range bacterial inhibitor cocktail

Recom ProteaseArrest[™] is a broad range, bacterial, 100X concentrated, ready-to-use protease inhibitor cocktail. Recom ProteaseArrest[™] offers greater protection for recombinant proteins expressed and purified from bacteria. Inhibits bacterial serine, cysteine, metallo- and other bacterial specific proteases including aminopeptidases and aspartic proteases.

Recom ProteaseArrest[™] cocktail does not use EDTA as its metalloprotease inhibitor as it would inhibit the activity of proteins that require divalent cations (Ca²⁺, Mg²⁺ or Mn²⁺) for their biological activity. In addition, EDTA would inhibit the purification of proteins using immobilized metal affinity chromatography (IMAC), for example His tagged or CBP tagged proteins. Recom ProteaseArrest[™] cocktail is compatible with immobilized metal affinity chromatography.

COMPOSITION

 668µM AEBSF, 1mM 1,10-Phenathroline, 1mM Benzamidine, 1mM Iodoacetamide, 1µM Pepstatin A, 1mM PMSF in DMSO

CITED REFERENCES

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Cat. No.	Description	Size
<u>786-376</u>	Recom ProteaseArrest [™] [100X]	1ml
<u>786-436</u>	Recom ProteaseArrest [™] [100X]	5ml

TCM ProteaseArrest

For use in tissue culture media

TCM ProteaseArrest[™] is a broad range, 200X concentrated, readyto-use protease inhibitor cocktail, sterile filtered for tissue culture media. TCM ProteaseArrest[™] inhibits a wide range of serine, cysteine and other specific proteases including aminopeptidases trypsin-like and acid proteases.

The inhibitor cocktail is designed to protect secreted proteins during cell culture for up to 48 hours.

COMPOSITION

 0.6µM Aprotinin, 58µM Bestatin, 28µM E-64, 10.5µM Leupeptin, 2.1µM Pepstatin A in DMSO

EATURES

- Broad range protease inhibitor cocktail
- · Stable for up to 48 hours in cell culture media
- Sterile filtered

Cat. No.	Description	Size
<u>786-238</u>	TCM ProteaseArrest [™] [200X]	1ml
<u>786-239</u>	TCM ProteaseArrest [™] [200X]	2ml

ProteCEASE[™]

A protease inhibitor cocktail for large scale preparative applications

ProteCEASE[™] is a dry format version of our ProteaseArrest[™] for large scale preparative applications and for those who prefer reconstitution prior to use.

ProteCEASE[™] is a superior general protease inhibitor cocktail that is suitable for purification from mammalian, plant, bacteria and yeast samples. The cocktail contains both irreversible and reversible protease inhibitors to inhibit serine, cysteine and other proteases. EDTA is an optional component and is for inhibiting metalloproteases.

The EDTA-free ProteCEASE[™] will maintain activity of proteins dependent on divalent cations and will not inhibit the purification of proteins with immobilized metal affinity chromatography (IMAC).

ProteCEASE[™] has been specifically developed for large scale preparative applications and is available in two vial sizes:

ProteCEASE[™]-50 for 50ml of lysis buffer.

ProteCEASE[™]-100 for 100ml of lysis buffer.

ProteCEASE[™]-50 is available in packs of 10 or 20 vials for 500ml and 1 liter total volume and ProteCEASE[™]-100 is available in packs of 10 for 1 liter total volume.

COMPOSITION

 668µM AEBSF, 0.3µM Aprotinin, 3µM Bestatin, 5mM EDTA (Optional), 5.25µM Leupeptin, 1mM PMSF

FEATURES

- High protease inhibition; >95% in mouse pancreas lysate
- Inhibits a wide variety of proteases, including cysteine, serine and metallo-proteases
- Designed for 50 and 100ml sample sizes
- Available with or without EDTA
- Performs with a wide range of samples, including animals, plants, yeast, bacteria and fungal lysates

APPLICATIONS

- · For large scale preparative protein purifications
- Inhibition of protease activity in protein preparations
- · Protection of proteins from proteolysis in such applications as electrophoresis, purification, storage, assays, and other applications

Protease Inhibitor Cocktails

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- Sun, X. et al (2002) Cancer Res. 62:6026 43. Ke Y and Theil F (2002) I Biol Chem 277 2373 44
- More refrences available at www.gbiosicences.com

Cat. No.	Description	Size
<u>786-326</u>	ProteCEASE [™] -50, EDTA free	10 vials
<u>786-326T</u>	ProteCEASE [™] -50, EDTA free	1 vial
<u>786-327</u>	ProteCEASE [™] -50, EDTA free	20 vials
<u>786-328</u>	ProteCEASE [™] -100, EDTA free	10 vials
<u>786-334</u>	ProteCEASE [™] -50, plus EDTA	10 vials
<u>786-335</u>	ProteCEASE [™] -50, plus EDTA	20 vials
<u>786-336</u>	ProteCEASE [™] -100, plus EDTA	10 vials

Individual Protease Inhibitors PROTEASE INHIBITOR COCKTAIL SELECTION GUIDE

				Concentration &
Product Name	Sample Type & key features	Inhibition Specificity	1X Composition	Available Sizes
			668µM AEBSF	
ProteaseARREST™	Fan dan and so a		0.3µM Aprotinin	100V DMCC solution
	For general use. EDTA is supplied in a	Serine, cysteine, calpain and metallo-	3µM Bestatin	100X DMS0 solution 24 x 100µl, 2ml, 5ml,
<u>I TOLCASCATINEST</u>	separate vial	proteases	5mM EDTA	10ml, 5 x 10ml
			5.25µM Leupeptin	20111, 0 / 20111
			1mM PMSF	
			668µM AEBSF	
		_	3µM Bestatin	
Postorial Protococ ADDESTIM	For bacterial cell extracts EDTA is supplied in a	Bacterial serine, cysteine and other bacterial specific proteases including	14µM E-64	100X DMSO solution
Bacterial ProteaseARREST™	separate vial	aminopeptidases and aspartic proteases	5mM EDTA	1ml, 5ml
			1µM Pepstatin A	
			1mM PMSF	
			668µm AEBSF	
			0.3µM Aprotinin	
	For mammalian tissue and	Mammalian serine, cysteine and other	3µM Bestatin	
	cell extracts	mammalian specific proteases including	14µM E-64	100X DMSO solution
Mammalian ProteaseARREST™	EDTA is supplied in a	aminopeptidases, trypsin-like and	5mM EDTA	1ml, 5ml
	separate vial	aspartic proteases	5.25µM Leupeptin	
			1µM Pepstatin A	
			1mM PMSF	
			668µM AEBSF	
			3µM Bestatin	
	For plant tissue and cell	Plant serine, cysteine and other	14µM E-64	
Plant ProteaseARREST™	extracts	plant specific proteases including	5.25µM Leupeptin	100X DMSO solution 1ml, 5ml
	EDTA is supplied in a	aminopeptidases, aspartic and metalloproteases	1µM Pepstatin A	
	separate vial	metanoproteases	2mM 1,10-Phenathroline	
			1mM PMSF	
			668µM AEBSF	
			14µM E-64	100X DMSO solution
Yeast/Fungal ProteaseARREST™	For yeast and fungal cell	Yeast and fungal serine, cysteine and	1µM Pepstatin A	
	extracts	metalloproteases	2mM 1,10-Phenathroline	1ml, 5ml
			1mM PMSF	
			668µM AEBSF	
		1mM 1 10 D	1mM 1,10-Phenathroline	
	For recombinant protein	Inhibits bacterial serine, cysteine, metallo- and other bacterial specific	1mM Benzamidine	100X DMSO solution
Recom ProteaseARREST™	isolation	proteases including aminopeptidases	1mM lodoacetamide	1ml, 5ml
		and aspartic proteases	1µM Pepstatin A	, -
			1mM PMSF	
			0.6µM Aprotinin	
	For protection of secreted	A wide range of serine, cysteine and	58µM Bestatin	200X Charila filtered
TCM ProteaseARREST™	proteins in tissue culture	other specific proteases including	28µM E-64	200X Sterile filtered DMSO solution
	media.	aminopeptidases trypsin-like and acid	10.5µM Leupeptin	1ml, 2ml
	Sterile filtered	proteases	2.1µM Pepstatin A	,
			340µM AEBSF	
			0.3µM Aprotinin	
FOCUS™ ProteaseARREST	Ferrera (4)		1.45µM Bestatin	
	For sensitive downstream	Reversible and irreversible inhibitors of		100X DMSO solution
	proteomic applications (i.e.	serine, cysteine, calpain and metallo- proteases	3.4µM Calpain Inhibitor I	1ml
	2DGE and mass spec)	protodolo	5.25µM Leupeptin	
			17µM Phosphoramidon	
			1mM PMSF	
			668µM AEBSF	lyophilized format with
	Dry Format with optional	Serine, cysteine, calpain and metallo- proteases	0.3µM Aprotinin	Lyophilized format with optional EDTA solution and solubilization
ProteCEASE™ 50			3µM Bestatin	
ProteCEASE™ 100			5mM EDTA	solution
			5.25µM Leupeptin	1, 10 & 20 vials
			1mM PMSF	

Table 1: Protease inhibitor cocktail selection guide.

AEBSF

4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride

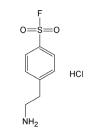


Figure 2: Structure of AEBSF.

Specificity: Specific irreversible inhibitor of serine proteases, including chymotrypsin, kallikrein, plasmin, thrombin and trypsin. A stable non-toxic alternative to PMSF.

Solubility: Water

Molecular weight: 239.7



ALLN

Calpain inhibitor I; N-[N-(N-acetyl-L-leucyl)-L-leucyl]-L-norleucine

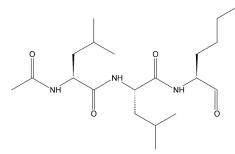


Figure 3: Structure of ALLN.

Specificity: Cell permeable peptide aldehyde inhibitor of calpain I and to a lesser extent calpain II. Also inhibits other neutral cysteine proteases, cathepsin B and L and the proteasome. Solubility: DMSO or ethanol

Molecular weight: 383.5

Cat. No.	Description	Size
<u>786-057</u>	<u>ALLN</u>	10mg

Individual Protease Inhibitors

Antipain, Dihydrochloride

[(S)-1-Carboxy-2-phenyl]-carbamoyl-arg-val-arginal

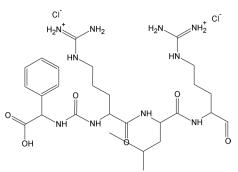


Figure 4: Structure of Antipain, Dihydrochloride.

Specificity: Inhibits Ca2+-dependent endopeptidases, including papain, trypsin-like serine proteases, some cysteine proteases and to a lesser extent plasmin. Higher specificity for trypsin and papain compared to leupeptin.

Solubility: Water, methanol and DMSO (Stock solution: 10mM) Molecular weight: 677.6

Cat. No.	Description	Size
786-045	Antipain dihydrochloride	5mg

Aprotinin



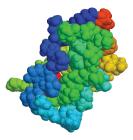


Figure 5: Rendering of Aprotinin.

Specificity: A broad range, competitive and reversible inhibitor of chymotrypsin, plasmin, trypsin, kallikrein and other serine proteases Solubility: Water (Stock solution: 10mM)

Molecular weight: 6512

A globular, monomeric protein chain. The sequence is RPDFC LEPPY TGPCK ARIIR YFYNA KAGLC QTFVY GGCRA KRNNF KSAED CMRTC GGA

- Zhao, N. et al (2016) Neogenin Facilitates the Induction of Hepcidin Expression by Hemojuvelin 45. in the Liver.J. Biol. Chem. 2016; 291:12322-12335.
- Beussman, K.M. et al (2015) Micropost arrays for measuring stem-cell derived cardiomyocyte contractility. Methods doi.org/10.1016/j.ymeth.2015.09.005 Beussman, K. M. et al (2015) Methods. DOI: 10.1016/j.ymeth.2015.09.005 46
- 47.
- 48 Shah, A.H. et al (2015) Scientific Reports. 5:11211

Cat. No.	Description	Size
<u>786-046</u>	<u>Aprotinin</u>	100mg
786-1245	<u>Aprotinin</u>	25mg

Individual Protease Inhibitors Bestatin

[(2S, 2R)-3-Amino-2-hydroxy-4-phenylbutanoyl]-L-leucine

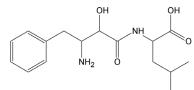


Figure 6: Structure of Bestatin.

Specificity: Competitive inhibitor of surface aminopeptidases, including aminopeptidase B (K_i=2nM), leucine aminopeptidase (K=20nM). Also inhibits aminopeptidases N; does not inhibit endoproteases.

Solubility: 5mg/ml in methanol or 1mg/ml in 0.15M NaCl Molecular weight: 308.4

Cat. No.	Description	Size
<u>786-047</u>	Bestatin	10mg

Chymostatin

N-[(S)-1-carboxy-isopentyl)-carbamoyl-alpha-(2iminohexahydro-4(S)-pyrimidyl]-L-glycyl-L-phenylalaninal

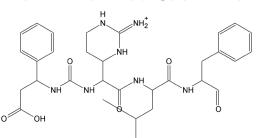


Figure 7: Structure of Chymostatin.

Specificity: Inhibits serine proteases having a chymotrypsin-like specificity, including α , β γ , and δ chymotrypsin, and most cysteine proteases including cathepsins B, H, L.

Solubility: DMSO

Molecular weight: 604.7



EDTA-Na2

Ethylenediamine-tetraacetic acid disodium salt dihydrate

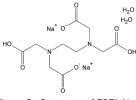


Figure 8: Structure of EDTA-Na,.

Specificity: Metal chelator that inhibits metalloproteases. Solubility: Water

Molecular weight: 372.24

Cat. No.	Description	Size
<u>786-050</u>	EDTA-Na ₂	100g
<u>RC-047</u>	EDTA-Na ₂	500g
<u>RC-048</u>	EDTA-Na ₂	1kg
<u>RC-051</u>	EDTA-Na	250g

E-64

L-trans-epoxysuccinyl-leucylamide-(4-guanido)-butane or N-[N-(L-trans-carboxyoxiran-2-carbonyl)-L-leucyl]agmatine

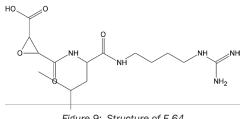


Figure 9: Structure of E-64.

Specificity: Irreversible inhibitor of cysteine proteases; does not inhibit serine proteases.

Solubility: DMSO (25mg/ml) and aqueous buffers (20mg/ml) Molecular weight: 357.4

CITED REFERENCES

González-Páez, G.E. and Wolan, D.W. (2012) J. Biol. Chem. 287:24412 1

Cat. No.	Description	Size
<u>786-049</u>	<u>E-64</u>	5mg
786-985	E-64	25mg

Leupeptin

Acetyl-leucyl-leucyl-arginal

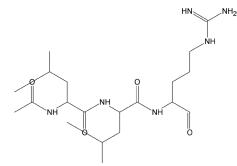


Figure 10: Structure of Leupeptin.

Specificity: Inhibits serine, plasmin, porcine kallikrein and cysteine proteases, including papain and cathepsin B. Does not inhibit chymotrypsin and thrombin.

Solubility: Water, ethanol, acetic acid and DMF

Molecular weight: 426.6

- Mihalas, B.P. et al (2015) Soc Reprod Fertil Suppl, 150:485 1.
- 2. Ma, L. et al (2015) J. Bacteriol. DOI: 10.1128/JB.00758-15
- З. Shah, A.H. et al (2015) Sci Rep. doi:10.1038/srep11211

Cat. No.	Description	Size
<u>786-051</u>	Leupeptin	25mg

Pepstatin

Isovaleryl-val-AHMHA-ala-AHMHA where AHMHA= (3S, 4S)-4-amino-3-hydroxy-6-methyl-heptanoic acid

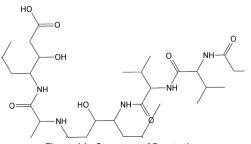


Figure 11: Structure of Pepstatin.

Specificity: A potent inhibitor of various aspartic proteases, including cathepsin D, renin, pepsin, bacterial aspartic proteases and HIV proteases.

Solubility: DMSO or methanol

Molecular weight: 685.9

CITED REFERENCES

- Huang, S. et al (2016) Protective Effect of Low-Intensity Pulsed Ultrasound on Memory Impairment and Brain Damage in a Rat Model of Vascular Dementia . RSNA. DOI: http://dx.doi. org/10.1148/radiol.2016160095
- Ayyub, A. et al (2015)Int. J. Biochem. Cell Biol.doi:10.1016/j.biocel.2015.11.006
- 3. Shah, A.H. et al (2015) Sci Rep. 5: e11211

Cat. No.	Description	Size
786-052	Pepstatin	25mg

Phosphoramidon

N-alpha-L-rhamnopyranosyloxy(hydroxyphosphinyl)-L-Leucyl-L-Tryptophan

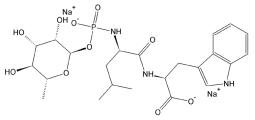


Figure 12: Structure of Phosphoramidon.

Specificity: Inhibits some metalloproteases, including thermolysin, collagenase and bacterial metalloproteases from *Bacillus subtilis*, *Streptomyces griseus* and *Pseudomonas aeruginosa* (metallo elastase).

Solubility: Water, methanol and DMSO Molecular weight: 543.5

CITED REFERENCES

 Hsieh, C. et al (2016) Persistent increased PKMζ in long-term and remote spatial memory. Neurobiol Learn Mem. DOI: 10.1016/j.nlm.2016.07.008

Cat. No	Description	Size
<u>786-05</u>	4 Phosphoram	idon 10mg

Individual Protease Inhibitors

PMSF

Phenylmethanesulfonyl fluoride

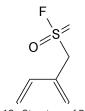


Figure 13: Structure of PMSF.

Activity: ≥99.9%

Specificity: Irreversible inhibitor of serine proteases, including trypsin and chymotrypsin. Also inhibits cysteine proteases and mammalian acetylcholinesterase.

Solubility: Methanol, ethanol and 2-propanol

Molecular weight: 174.2

- CITED REFERENCES
- Yalpani, N. et al (2017) An Alcaligenes strain emulates Bacillus thuringiensis producing a binary protein that kills corn 3 rootworm through a mechanism similar to Cry34Ab1/Cry35Ab1 Supplementary Methods
- Shane, M.W. and Plaxton, C.W. (2015) Extraction of Intracellular and Cell Wall Proteins from Leaves and Roots of Harsh Hakea. Bio Protoc. 5:2
- 3. Blackler, RJ. et al (2015) Glycobiology. doi: 10.1093/glycob/cwv093

Cat. No.	Description	Size
<u>786-055</u>	<u>PMSF</u>	5g
<u>786-787</u>	<u>PMSF</u>	25g
<u>786-788</u>	<u>PMSF</u>	100g

PROTEASE INHIBITOR SET

100X concentrated protease inhibitor selection

Contains 12 ready-to-use individual protease inhibitors for characterization of protease activity.

Each protease inhibitor is supplied in a ready-to-use solution at a 100X concentration. The 1X concentration of the protease inhibitors is designed to give >90% inhibition in crude tissue extracts. Various concentrations and/or combinations of protease inhibitors may be used to inhibit a broad spectrum of protease activity.

The Protease Inhibitor Set can be used to design specific protease inhibitor cocktails, supplement existing cocktails or to screen for specific protease classes.

Each set contains the following protease inhibitors. See previous section for their specificities and other information:

- AEBSF
- ALLN
- Antipain, dihydrochloride
- Aprotinin
- Bestatin
- · Chymostatin
- E-64
- EDTA-Na₂
- Leupeptin
- Pepstatin
 - Phosphoramidon
 - PMSF

- 1. Elmi, A. et al (2015) Cell. Microbiol.DOI: 10.1111/cmi.12534
- 2. Silva, B. D. et al (2015) Exp. Cell Res. DOI: 10.1016/j.yexcr.2015.08.021
- Paliwal, D. et al (2014) J Gen Virol. 95:1689
 Narayanaswamy, R. et al (2014) Mol Cell Biol. 35:2553
- Siddappa, D. et al (2014) Mol Reprod Dev. 81:655

Cat. No.	Description	Size
		40.05

<u> 186-201</u>	Protease Inhibitor Set	12 x 25µl

Individual Protease Inhibitors

INHIBITOR SELECTION GUIDE

Cat. No.	Protease Inhibitor	Specificity	Solubility	Molecular Weight	Quantity Supplied
<u>786-053</u>	AEBSF 4-(2-Aminoethyl)benzenesulfonyl fluo- ride hydrochloride	Specific irreversible inhibitor of serine proteases, including chymotrypsin, kallikrein, plasmin, thrombin and trypsin. A stable non-toxic alternative to PMSF.		239.7	1g
<u>786-057</u>	ALLN Calpain inhibitor I; N-{N-{N-Acetyl-L- leucyl}-L-leucyl]-L-norleucine	Cell permeable peptide aldehyde inhibitor of calpain I and to a lesser extent calpain II. Also inhibits other neutral cysteine proteases, cathepsin B and L and the proteasome.	DMSO Ethanol	383.5	10mg
<u>786-045</u>	Antipain, Dihydrochloride [(S)-1-Carboxy-2-Phenyl]-carbamoyl- Arg-Val-arginal	Inhibits Ca ²⁺ -dependent endopeptidases, including papain, trypsin-like serine proteases, some cysteine proteases and to a lesser extent plasmin. Higher specificity for trypsin and papain compared to leupeptin.	H ₂ O Methanol DMSO	677.6	5mg
<u>786-046</u>	Aprotinin Bovine pancreatic trypsin inhibitor	A broad range, competitive and reversible inhibitor of chymotrypsin, plasmin, trypsin, kallikrein and other serine proteases.	H ₂ 0	6512	100mg
<u>786-047</u>	Bestatin [(2S, 2R)-3-Amino-2-hydroxy- 4-Phenylbutanoyl]-L-Leucine	Competitive inhibitor of surface aminopeptidases, including aminopeptidase B (K_1 =2nM), leucine aminopeptidase (K_1 =20nM). Also inhibits aminopeptidases N; does not inhibit endoproteases.	Methanol (<5mg/ml) NaCl [0.15M] (<1mg/ml)	308.4	10mg
<u>786-048</u>	Chymostatin N-[(S)-1-carboxy-isopentyl)-carbam- oyl-alpha-(2-iminohexahydro-4(S)- pyrimidyl]-L-glycyl-L-phenylalaninal	Inhibits serine proteases having a chymotrypsin-like specificity, including α , β γ , and δ chymotrypsin, and most cysteine proteases including cathepsins B, H, L.	DMSO	604.7	5mg
<u>786-049</u>	E-64 L-trans-epoxysuccinyl-leucylamide-(4- guanido)-butane or N-[N-(L-trans- carboxyoxiran-2-carbonyl)-L-leucyl]- agmatine	Irreversible inhibitor of cysteine proteases; does not inhibit serine proteases.	DMSO (25mg/ml) Aqueous buffers (20mg/ml)	357.4	5mg
<u>786-050</u>	EDTA-Na2 Ethylenediamine-tetraacetic acid diso- dium salt dihydrate	Metal chelator that inhibits metalloproteases.	H ₂ 0	372.24	100g
<u>786-051</u>	Leupeptin Acetyl-leucyl-leucyl-arginal	Inhibits serine, plasmin, porcine kallikrein and cysteine proteases, including papain and cathepsin B. Does not inhibit chymotrypsin and thrombin.	H ₂ O Ethanol Acetic Acid DMF	426.6	25mg
<u>786-052</u>	Pepstatin Isovaleryl-Val-Val-AHMHA-Ala-AHMHA where AHMHA= (3S, 4S)-4-amino-3-hy- droxy-6-methyl-heptanoic acid	A potent inhibitor of various aspartic proteases, including cathepsin D, renin, pepsin, bacterial aspartic proteases and HIV proteases.	Methanol	685.9	25mg
<u>786-054</u>	Phosphoramidon N-alpha-L-rhamnopyranosyloxy(hydroxy phosphinyl)-L-Leucyl-L-Tryptophan	Inhibits some metalloproteases, including thermolysin, collagenase and bacterial metalloproteases from <i>Bacillus subtilis</i> , <i>Streptomyces griseus</i> and <i>Pseudomonas aeruginosa</i> (metallo elastase).	H ₂ O Methanol DMSO	543.5	10mg
<u>786-055</u>	PMSF Phenylmethanesulfonyl fluoride	Irreversible inhibitor of serine proteases, including trypsin and chymotrypsin. Also inhibits cysteine proteases and mammalian acetylcholinesterase.	Methanol Ethanol 2-propanol	174.2	5g

Table 2: Protease inhibitor selection guide.

Protease Assays & Screening Systems

ProteSEEKER[™]

Identify destructive proteases

ProteSEEKER[™] identifies specific types of proteases with a panel of twelve protease inhibitors and a sensitive colorimetric protease screening assay. It gives researchers the ability to screen their protein samples and establish which specific class of proteases are present and therefore design a highly specific protease inhibitor cocktail using the minimal number of protease inhibitors. Alternatively, ProteSEEKER[™] can be used to test existing protease inhibitor cocktails and identify their inadequacies and therefore supplement in additional protease inhibitors.

ProteSEEKER[™] protease screening assay consists of a ready-touse dye-labeled protein, which is digested by proteases to release dye-labeled peptides. The absorbance of which is measured for determination of protease activity. The inhibitors are supplied at a 100X concentration and the 1X concentration provides >90% inhibition in most biological samples. ProteSEEKER[™] kit is sufficient for 50 assays:

CITED REFERENCES

- Soman, K. et al (2017) Activation of Human Peripheral Blood Eosinophils by Cytokines in a Comparative Time-Course Proteomic/Phosphoproteomic Study. J. Proteome Res. DOI: 10.1021/ acs.jproteome.6b00367
- 2. Chang, W.W.P. et al (2005) Amer. Biotech. Lab



Protease Screening Kit

Detect protease activity in your sample

Provides a simple and quick method for testing your samples for proteolysis. Simply incubate your sample in the reagent provided and obtain results. The kit uses dye-labeled protein conjugate as protease substrate, which allows nanogram level detection. The absorbance of dye-labeled peptide is measured at 574nm for determination of protease activity. The kit is sufficient for 50 assays in a microwell format.

CITED REFERENCES

- Ermolova, N. et al (2011) Hum Mol Genet. 20:3331
 Pankow, K. et al (2007) Circ. Res. 101:875
 - Cat. No.DescriptionSize786-137Protease Screening Kit50 Assays

Protease Assay Kit

For assay of protease activity

Designed for the determination of proteases present in a protein sample, using a dye-labeled protein substrate.

The proteases present in the sample of interest will digest the protein substrate and release dye labeled peptides. The absorbance of the dye-labeled peptide is measured at 570nm for determination of protease activity.

Mass spectrometry grade trypsin is supplied as a general protease standard; however, other specific protease standards can also be used. The trypsin is an ultra-pure trypsin from bovine pancreas, modified by methylation followed by TPCK treatment and is extremely resistant to autolysis.

The kit components are sufficient for 50 assays in a microtiter plate format or 0.5ml assay tubes.

APPLICATIONS

 Determination of protease activity in biological samples, with nanogram detection levels

- 1. Marine, E.J. et al (2016) Synthesis and Self-Assembly of Bundle-Forming α-Helical Peptide–Dendron Hybrids. Biomacromolecules. 17:336
- 2. Zhang, D. et al (2015) BMC Oral Health. 15:128
- 3. Tripathi, T. et al (2015) Receptors Clin Inv. doi: 10.14800/rci.917

Cat. No.	Description	Size
<u>786-028</u>	Protease Assay Kit	50 Assays

Protease Assays & Screening Systems

Fluoro[™] Protease Assay

A fluorometric, quantitative protease assay

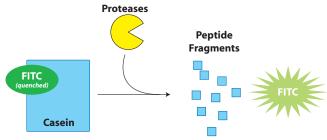


Figure 14: Fluoro[™] Protease Assay Scheme.

The *Fluoro*[™] Protease Assay Kit is designed for the quantitative determination of proteases present in a protein sample. The assay uses fluorescein isothiocyanate (FITC)-labeled casein as a general protease substrate. The fluorescein label on the FITC-casein is highly quenched. When the proteases present in the sample of interest digest the FITC-casein substrate into smaller peptides, the quenching of the fluorescence label is relieved and the fluorescence of the substrate is increased. The fluorescence of the FITC-labeled peptide is measured with excitation at 485nm and emission at 535nm to determine protease activity. The kit detects picogram level of proteases present in the sample.

The kit is supplied with mass spectrometry grade trypsin for use as a general protease control; however, other specific protease standard controls can be used. The trypsin is an ultra-pure trypsin from porcine pancreas, modified by methylation followed by TPCK treatment and is extremely resistant to autolysis. The kit components are sufficient for 1,000 assays in a microtiter plate format.

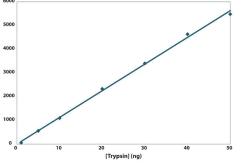


Figure 15: The graph depicts the linear response of the Fluoro[™] Protease Assay with increasing concentrations of trypsin protease.

APPLICATIONS

· Quantitative fluorescence protease assay

CITED REFERENCES

- Burlet, E. et al (2017) Evaluation of the Potency, Neutralizing Antibody Response, and Stability of a Recombinant Fusion Protein Vaccine for Streptococcus pyogenes. AAPS J. DOI: 10.1208/ s12248-017-0069-5
- Guo, X. et al (2016) Proteins in the Cocoon of Silkworm Inhibit the Growth of Beauveria bassiana. PLoS One. doi.org/10.1371/journal.pone.0151764
- Li, Y. et al (2014) Insect Biochem Mol Biol. doi:10.1016/j.ibmb.2014.11.006
 Gwyther, C.L. et al (2014) Environ. Tech. DOI:10.1080/09593330.2014.885585
- Gwyther, C.L. et al (2014) Environ. Tech. DOI:10.1080,
 Li, Y. et al (2012) Insect Biochem. Molec. 42:766



Protease Assay Substrates

Resorufin and FITC-casein protease substrates

Resorufin-case in protease substrate is a colorimetric substrate that when treated with proteases releases resorufin that has an absorbance of 570nm. Supplied lyophilized.

A fluorescent (fluorescein isothiocyanate (FITC)) substrate that when treated with proteases releases FITC that has an excitation at 485nm and emission at 535nm. Supplied lyophilized.

CITED REFERENCES

 Wang, D. et al (2016) Serine protease P-IIc is responsible for the digestion of yolk proteins at the late stage of silkworm embryogenesis. Insect Biochem Mol Biol. doi:10.1016/j. ibmb.2016.03.003.

Cat. No.	Description	Size
<u>786-321</u>	Resorufin-Casein protease substrate	4mg
<u>786-322</u>	FITC-Casein protease substrate	1mg

Phosphatase Inhibitor Cocktails

The PhosphataseArrest™ phosphatase inhibitor cocktails are ready-to-use 100X solutions that are simply added to your extraction buffers or samples. Compatible with most phosphatase assays and no resuspension required.

PhosphataseArrest[™] I

A broad spectrum phosphatase inhibitor cocktail consisting of five phosphatase inhibitors that target serine/threonine specific, tyrosine specific and dual specificity phosphatases.

PhosphataseArrest[™] I is a stablized solution of sodium fluoride, sodium orthovanadate, sodium pyrophosphate, β-glycerophosphate & sodium molybdate.

Phosphatase Inhibitor	M.W.	Target Phosphatases
Sodium fluoride	42.0	Acid phosphatases
Sodium Orthovanadate	183.9	Tyrosine phosphatase, Alkaline phosphatase
Sodium Pyrophosphate	221.94	Serine/Threonine phosphatases
β-Glycerophosphate	306.1	Serine/Threonine phosphatases
Sodium Molybdate	205.92	Acid Phosphatase

PhosphataseArrest[™] II

A phosphatase inhibitor cocktail consisting of five phosphatase inhibitors that target acid, alkaline and tyrosine phosphatases.

PhosphataseArrest[™] II contains optimized concentrations of sodium fluoride, sodium tartrate, sodium orthovanadate, imidazole & sodium molybdate.

Phosphatase Inhibitor	M.W.	Target Phosphatases
Sodium fluoride	42.0	Acid phosphatases
Sodium Orthovanadate	183.9	Tyrosine phosphatases, Alkaline phosphatases
Sodium Tartrate	230.08	Acid phosphatases
Imidazole	68.08	Alkaline phosphatases
Sodium Molybdate	205.92	Acid Phosphatases

PhosphataseArrest[™] III

A phosphatase inhibitor cocktail consisting of three phosphatase inhibitors, that target alkaline and serine/threonine phosphatases.

PhosphataseArrest[™] III is a stable, convenient solution of cantharidin, p-bromotetramisole oxalate and calyculin.

Phosphatase Inhibitor	M.W.	Target Phosphatases
Cantharidin	196.2	Serine/Threonine phosphatases
p-Bromotetramisole Oxalate	373.23	Alkaline phosphatases
Calyculin	1009.17	Serine/Threonine phosphatases

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Selection Guide for Phosphatase Inhibitor Cocktails

Cat. No.	Description	Target Phosphatases	Size
<u>786-450</u>	PhosphataseArrest [™] I [100X]	Serine/Threonine Tyrosine Dual Specificity	1ml
<u>786-647</u>	PhosphataseArrest [™] I [100X]	Serine/Threonine Tyrosine Dual Specificity	24 x 100ul
<u>786-782</u>	PhosphataseArrest [™] I [100X]	Serine/Threonine Tyrosine Dual Specificity	2ml
<u>786-783</u>	PhosphataseArrest [™] I [100X]	Serine/Threonine Tyrosine Dual Specificity	5ml
<u>786-784</u>	PhosphataseArrest [™] I [100X]	Serine/Threonine Tyrosine Dual Specificity	10ml
<u>786-451</u>	PhosphataseArrest [™] II [100X]	Acid Alkaline Tyrosine	1ml
<u>786-452</u>	PhosphataseArrest [™] III [100X]	Alkaline Serine/Threonine	1ml

Phosphatase Assays

Phosphatase Assay

A pNPP based assay for simple phosphatase estimation

The Phosphatase Assay kit is designed to measure the activity of phosphatases in biological samples and to screen for agonists and inhibitors of phosphatases.

The Phosphatase Assay kit uses para-nitrophenyl phosphate (pNPP), a chromogenic substrate for most phosphatases, including alkaline phosphatases, acid phosphatases, protein tyrosine phosphatases and serine/threonine phosphatases.

The phosphatases remove the phosphate group to generate p-nitrophenol, which is deprotonated under alkaline conditions to produce p-nitrophenolate that has strong absorption at 405nm.

The kits components are sufficient for performing up to 1000 assays in 96-well plate format and easily adaptable to cuvettes or 384-well plates.

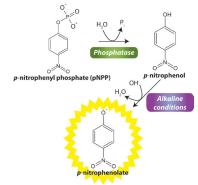


Figure 16: Scheme of Phosphatase Assay.

FEATURES

- A colorimetric, pNPP based assay
- · Measure phosphatase activity in biological samples
- Screen for phosphatase agonists and inhibitors

APPLICATIONS

- For the quantification of phosphatase activity
- To screen for agonists and inhibitors of phosphatases

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Estimation of phosphates in phosphoproteins.

PhosphoQuant[™] is specifically designed for quick and reliable determination of whether a purified protein is phosphorylated and the extent of phosphorylation. The assay is based on the alkaline hydrolysis of phosphates from seryl and threonyl residues in phosphoproteins and the subsequent quantification of the released phosphate with a Molybdate dye.

Cat. No.	Description	Size
<u>786-256</u>	<u>PhosphoQuant</u> [™]	400 assays

Protease-PhosphataseArrest™ [100X]

Protease-PhosphataseArrest[™] provides full protection of protein samples from proteases and phosphatases released during the preparation of cell and tissue lysates.

Cat. No.	Description	Size
<u>786-870</u>	Protease-PhosphataseArrest [™] _[100X]	For 100ml
<u>786-871</u>	Protease-PhosphataseArrest [™] _[100X]	For 200ml
<u>786-872</u>	Protease-PhosphataseArrest [™] [100X]	For 500ml
<u>786-889</u>	Protease-PhosphataseArrest [™] [100X]	For 240ml

PhosphoQuant[™]

Proteases

MASS SPECTROMETRY GRADE PROTEASE

Trypsin for Mass Spectrometry

Trypsin is a serine endopeptidase that specifically cleaves peptide bonds on the carboxy side of s-aminoethyl cysteine, arginine and lysine residues. Typically there is little or no cleavage at arginylproline and lysyl-proline bonds.

Trypsin undergoes autolysis, producing trypsin fragments that interfere with sequence analysis. G-Biosciences' mass spectrometry grade trypsin is a chemically modified trypsin that is enzymatically active and yet resistant to autolysis. Mass spectrometry grade trypsin is methylated, TPCK treated and quality tested for mass spectrometry.

Unlike other trypsin preparations, mass spectrometry grade trypsin is highly stable, maintaining its activity in severe denaturing buffers and as a result, is shipped without requiring dry ice and can be stored for a long period without any loss of activity.

We supply two sources of mass spectrometry grade trypsin, either bovine or porcine. For mass spectrometry sequence analysis, mass spectrometry grade trypsin to protein ratio of 1:20 to 1:100 is recommended. For convenience, mass spectrometry grade trypsin is supplied in 20µg, 100µg and 200µg vials with a specific resuspension buffer.

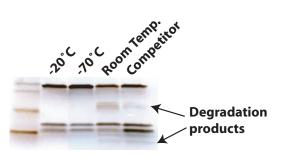


Figure 17: MALDI-TOF Mass Spectrum of casein digested with our mass spectrometry grade trypsin.

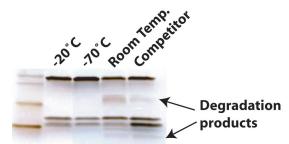


Figure 18: Mass spectrometry grade trypsin is highly stable. Stored at -20°C, -70°C and room temperature for six months and then resuspended and analyzed by SDS-PAGE and stained with FOCUS FastSilver[™]; For a comparison, a competitor's trypsin was resuspended according to the manufacturer's protocol and an equivalent amount was analyzed. Only our mass spectrometry grade trypsin stored at room temperature and the competitor's trypsin showed degradation products.

FEATURES

- Ultra pure porcine or bovine trypsin
- · Modified by methylation and TPCK treatment
- · Resistant to autolysis and degradation
- For sequence analysis and mass spectrometry applications
- Stable at ambient temperature and suitable for long term storage
- Specific activity >10,000U/mg protein

APPLICATIONS

- · Digestion of proteins for sequence and peptide fragment analysis
- · Suitable for sequencing and mass spectrometry applications

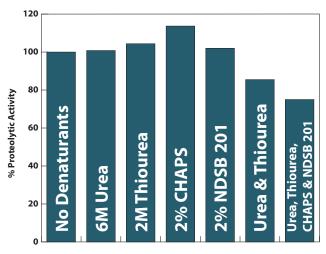


Figure 19: Proteolytic activity of mass spectrometry grade trypsin is maintained in the presence of various severe denaturing buffers as assessed by our Protease Screening Kit.

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Cat. No.	Description	Size
<u>786-245</u>	Trypsin, Mass Spectrometry Grade (Porcine)	5 x 20µg
<u>786-245B</u>	Trypsin, Mass Spectrometry Grade (Bovine)	5 x 20µg
<u>786-687</u>	Trypsin, Mass Spectrometry Grade (Porcine)	100µg
<u>786-687B</u>	Trypsin, Mass Spectrometry Grade (Bovine)	100µg
<u>786-688</u>	Trypsin, Mass Spectrometry Grade (Porcine)	200µg
<u>786-690</u>	Trypsin, Mass Spectrometry Grade (Porcine)	5 x 100µg
<u>786-693</u>	Trypsin, Mass Spectrometry Grade (Porcine)	5 x 200µg

GENERAL PROTEASE

Proteinase K

Specificity: Non-specifc, broad spectrum serine protease that is isolated from the saprophytic fungus *Tritirachium album*. **Solubility:** Highly soluble (>50mg/ml)

Molecular weight: 28.93 kDa

Cat. No.	Description	Size
<u>786-043</u>	Proteinase K, lyophilized	100mg
<u>786-044</u>	Proteinase K, lyophilized	5 x 100mg
<u>786-035</u>	Proteinase K, lyophilized	250mg
<u>786-064</u>	Proteinase K, lyophilized	1mg
<u>786-065</u>	Proteinase K, lyophilized	5g

Trypsin, Lyophilized

A general use trypsin

Purified from bovine pancreas, 1X crystallized, dialyzed against 1mM HCl and lyophilized. >150 TAME untis/ mg protein. One Unit hydrolyzes 1 μ mole of p-toluene-sulfonyl-L-arginine methyl ester (TAME) per minute at 25 °C, pH 8.2, in the presence of 10mM calcium.

CITED REFERENCES

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Cat. No.	Description	Size
<u>786-262</u>	<u>Trypsin</u>	1g

Enterokinase (Recombinant)

Enterokinase (Recombinant) is highly purified recombinant bovine enterokinase obtained from E. Coli. Enterokinase is highly specific serine protease that hydrolysis peptide bond at the carboxyl side of lysine residue preceded by four aspartic acids (FLAG-tag).

FEATURES

- Animal free source of origin, therefore no any other contaminating proteases.
- Highly purified recombinant enterokinase that cleaves specifically after lysine preceded by four aspartic acid residues.

APPLICATIONS

 Enterokinase is used for removal of Flag-Tag from fusion proteins with the FLAG-tag.

Cat. No.	Description	Size
<u>786-1246</u>	Enterokinase	20 U
<u>786-1247</u>	Enterokinase	100 U
786-1248	Enterokinase	5 x 100 U

SEQUENCING GRADE PROTEASE

SG-Chymotrypsin[™]

Hydrolysis of peptide bonds on the carboxy side of tyrosine, phenylalanzine & tryptophan

A serine endopeptidase, which predominantly cleaves peptide bonds on the carboxy side of tyrosine, phenylalanine and tryptophan. In addition, chymotrypsin has a low catalytic activity against the carboxy side of leucine, methionine, alanine, aspartic and glutamic acids. It is therefore recommended to always use the shortest digestion time possible.

SG-Chymotrypsin[™] is first treated with TLCK to inhibit trypsin that may be present and then subjected to an extensive purification process to remove contaminating protease and chymotryptic autolysis by-products. The highly purified enzyme is then chemically modified to increase its resistance to autolysis and stability.

Use at a ratio of 1:200 to 1:50, by weight, in a standard digestion buffer. Incubate at 25-30 $^{\circ}$ C for 1 to 10 hours, but can be extended to 24 hours.

Cat. No.	Description	Size
<u>786-13</u>	<u>SG-Chymotrypsin</u> [™]	2 x 25µg

SG-Lysine-C[™]

Cleaves peptide bonds at the carboxy side of lysine

An endopeptidase, from Lysobacter enzymogenes, is a serine protease highly specific in cleaving peptide bonds at the carboxy side of lysine. Highly purified preparations of SG-Lysine-C^M are chemically modified making the enzyme resistant to autolysis and stabilizing its enzymatic activity.

SG-Lysine-C^M is supplied lyophilized in 5µg vials. The enzyme is typically reconstituted to a concentration of 0.25µg/ml. For fragmentation, the enzyme is added to the sample protein in a ratio of 1:100 to 1:20 (enzyme to protein, by weight) in a standard digestion buffer.

Cat. No.	Description	Size
<u>786-14</u>	<u>SG-Lysine-C</u> [™]	2 x 5µg

SG-Glutamic-C[™]

Cleaves peptide bonds at the carboxy side of either aspartic or glutamic acid

A serine endopeptidase, from *Staphylococcus aureus V8*, that is highly specific for the cleavage of peptide bonds at the carboxy side of either aspartic or glutamic acid, depending on the buffer used. In Tris-HCl buffer, in particular in the absence of phosphate ions, the enzyme is specific for the glutamyl site. Recommended buffers for fragmentation of proteins using this enzyme are 50mM Tris-HCl, pH 8.0 or bicarbonate buffer. Highly purified preparations of SG-Glutamic-C[™] are chemically modified making the enzyme both resistant to autolysis and stabilizes its enzymatic activity.

SG-Glutamic-C[™] is supplied lyophilized in 10µg vials. The enzyme is typically reconstituted to a concentration of 0.5µg/ml and commonly used at a ratio of 1:100 to 1:20 (enzyme to protein, by weight) in a standard digestion buffer.

CITED REFERENCES

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Cat. No.	Description	Size
<u>786-15</u>	<u>SG-Glutamic-C</u> [™]	2 x 10µg

SG-Arginine-C[™]

Endopeptidase for the specific hydrolysis of the carboxy peptide bond of arginine

An endopeptidase (Clostripain, from *Clostridium histolyticum*) specifically hydrolyzes the carboxy peptide bond of Arginine. SG-Arginine-C[™] has been modified chemically by a propriety process to render the enzyme resistant to autolysis and stabilize enzymatic activity. In addition, as a sulfhydryl enzyme, SG-Arginine-C[™] is susceptible to inactivation by oxidation and as a result requires reducing agents for protection. The enzyme also requires calcium ion for maximal activity. A special reconstitution buffer is supplied, which contains reducing agents and activators to maintain enzyme activity.

SG-Arginine-CTM is supplied lyophilized in an activated form in 5µg vials and can be reconstituted to a concentration of 0.25µg/ml by addition of 20µl per vial of the supplied reaction buffer. For fragmentation the enzyme is added to the sample protein in a ratio of 1:100 to 1:20 (enzyme to protein, by weight).

CITED REFERENCES

 Friedrick, R.P at al (2016) Tissue Plasminogen Activator Binding to Superparamagnetic Iron Oxide Nanoparticle–Covalent Versus Adsorptive Approach.Nanoscale Res Lett.DOI: 10.1186/ s11671-016-1521-7

Cat. No.	Description	Size
<u>786-11</u>	<u>SG-Arginine-C</u> [™]	2 x 5µg

SG-Carboxypeptidase B (Recombinant)™

SG-Carboxypeptidase B (Recombinant)™is the rat carboxypeptidase B expressed in E. coli. Carboxypeptidase B specifically hydrolyses basic amino acids including lysine, arginine and histidine from the C-terminal end of polypeptides.

FEATURES

- Animal free source of origin, therefore no any other contaminating proteases:
- No protease inhibitors are present during preparation of SG-Recombinant Carboxypeptidase B[™]
- High Purity: HPLC grade; single band on SDS-PAGE; no other contaminating proteases such as chymotrypsin and carboxypeptidase A. Less than 10ppm of recombinant trypsin.

APPLICATIONS

 SG-Carboxypeptidase B (Recombinant)[™] is used in sequencing protein and peptides.

Cat. No.	Description	Size
<u>786-1249</u>	<u>SG-Carboxypeptidase B</u> (Recombinant)™	0.1 mg
<u>786-1250</u>	<u>SG-Carboxypeptidase B</u> (Recombinant)™	1 mg

SG-Chymotrypsin (Human, Recombinant)™

SG-Chymotrypsin (Human, Recombinant)[™] is recombinant human chymotrypsin expressed in E. coli and purified by HPLC method.

Chymotrypsin hydrolysis at the carboxyl side of aromatic amino acid residues including Tyrosine, phenylalanine and Tryptophan. Cleavage occurs at lower rate at Leucine and methionine residues.

FEATURES

- Animal free source of origin, therefore no any other contaminating proteases.
- High purity:>95%, purified with HPLC

APPLICATIONS

 Chymotrypsin is used peptide mapping (mass spectrometry), fingerprinting and sequence analysis alone or along with other proteases.

Cat. No.	Description	Size
786-1251	<u>SG-Chymotrypsin (Human,</u> <u>Recombinant)™</u>	0.1 mg
786-1252	<u>SG-Chymotrypsin (Human,</u> <u>Recombinant)™</u>	1 mg

Trypsin (Human, Recombinant)

Trypsin is a serine protease that cleaves peptides on C-terminal end of lysine and arginine amino acid residues. The pH optimum of trypsin is pH 7.0-8.0. Trypsin is inhibited by serine protease inhibitors including TLCK (N-p-tosyl-L-lysine chloromethyl ketone), PMSF (phenylmethanesulfonyl fluoride), benzamidine, soybean trypsin inhibitor, and ovomucoid

Trypsin (Human, Recombinant) is genetically engineered human trypsin expressed in E. coli and purified by high pressure liquid chromatography. It has animal free source of origin, so is virus free and also it has no other contaminating proteases such as chymotrypsin and carboxypeptidase. No protease inhibitor such as PMSF involved in its preparation.

FEATURES

- Animal free source of origin: Recombinant human trypsin expressed in E. coli. Free from contaminating proteases such as chymotrypsin and carboxypeptidase A and viruses.
- High purity: ≥ 95%; purified by high pressure liquid chromatography

APPLICATIONS

- Trypsin (Human, Recombinant) can be used to make celldissociation reagents
- · It can be used for digestion of peptide and proteins for sequencing.

Cat. No.	Description	Size
<u>786-1253</u>	Trypsin (Human, Recombinant)	1 mg
<u>786-1254</u>	Trypsin (Human, Recombinant)	5 mg
<u>786-1255</u>	Trypsin (Human, Recombinant)	50 mg

Immobilized Proteases

IMMOBILIZED PROTEASE

Immobilized Trypsin

Immobilized Trypsin is TPCK treated trypsin immobilized on 4% agarose that eliminates the contamination of protein digests by the trypsin. The immobilized trypsin is readily removed by separating the agarose from the digestion solution.

Trypsin is a serine endopeptidase that specifically cleaves peptide bonds on the carboxy side of s-aminoethyl cysteine, arginine and lysine residues and typically there is little or no cleavage at arginylproline and lysyl-proline bonds. The distribution of these residues in proteins allows trypsin digestion to produce peptides that are readily identified by mass spectrometry.

Native trypsin is prone to autolysis that results in pseudotrypsin, which exhibits a broader proteolytic specificity (a chymotrypsin like activity) and trypsin fragments that interfere with sequence analysis.

The trypsin is TPCK treated to inactive the interfering chymotrypsin activity and the resulting protein is affinity purified.

Immobilzed Trypsin is supplied as a 50% slurry containing glycerol and sodium azide as a preservative.

FEATURES

- · Eliminate contamination with trypsin
- Source: Bovine
- Activity: ≥200 TAME units/mI resin
- Support: 4% Cross-linked Agarose



Immobilized Papain

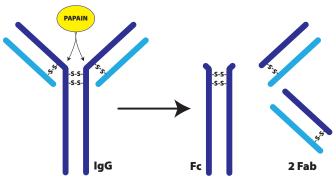


Figure 20: Digestion of Immunglobulin G with Papain.

A cysteine protease enzyme (EC 3.4.22.2) immobilized on 4% agarose, cleaves immunoglobulin G antibody molecules in the hinge region, generating three ~50kDa fragments; two Fab domains and a Fc domain. The papain-digested antibody is unable to promote agglutination, precipitation, opsonization, and lysis.

FEATURES

- Generate Fc and Fab from IgG
- · Eliminates contamination with papain enzyme
- Can be used in virtually all scenarios using free papain

CITED REFERENCES

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- Vol #: Structure/2016/24 3. Zhang, H. amd Lin, S. (2003) J. Phycol. 39:1160

Cat. No.	Description	Size
<u>786-790</u>	Immobilized Papain	5ml Resin

Immobilized Pepsin

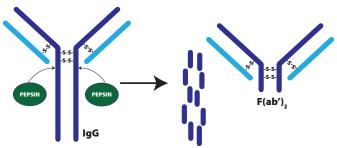


Figure 21: Digestion of Immunglobulin G with Pepsin.

A proteolytic enzyme immobilized on 4% agarose that is routinely used for the generation of $F(ab')_2$ fragments from immunoglobulin G (lgG). The pepsin has the ability to cleave the heavy chains near the hinge region. One or more of the disulfide bonds that join the heavy chains in the hinge region are preserved, so the two Fab regions of the antibody remain joined together, yielding a divalent molecule (containing two antibody binding sites), hence the designation $F(ab)_2$. The light chains remain intact and attached to the heavy chain, whereas the Fc fragment is digested into small peptides.

The Immobilized Pepsin offers the distinct advantage of eliminating enzyme contamination of the $F(ab)_2$ fragments.

FEATURES

- Generate F(ab)₂ fragments
- Eliminate contaminating pepsin enzyme
- · Can be used in virtually all scenarios using free pepsin

Cat. No.	Description	Size
<u>786-791</u>	Immobilized Pepsin	5ml Resin

Immobilized Ficin

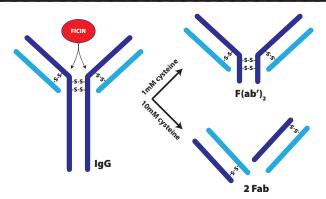


Figure 22: Digestion of Immunglobulin G with Ficin.

Ficin (or Ficain) is a cysteine protease enzyme (EC 3.4.22.3) isolated from fig latex is that has the endopeptidase activity to cleave immunoglobulin G molecules in the hinge reason. Ficin is typically used to cleave mouse IgG_1 as this is difficult to cleave with papain and pepsin. In the presence of 1mM or 10mM cysteine, ficin generates $F(ab)_2$ and Fab fragments respectively. Immobilized Ficin is a convenient reagent for producing Fab and $F(ab)_2$ fragments as it avoids the need to remove the ficin enzyme after digestion.

FEATURES

- Generate Fab and F(ab'), fragments
- For digestion of mouse IgG1
- Eliminates contamination by Ficin

Cat. No.	Description	Size
786-793	Immobilized Ficin	5ml Resin

Protein Sequencing Analysis Tools

InGel[™] Silver

In gel digestion of proteins in silver stained gels

InGel[™] Silver provides a complete set of reagents for the in gel tryptic digestion and extraction of peptides for mass spectrometry (MALDI and LC MS/MS). The kit is specifically designed for use with silver stained protein spots/bands.

The protein spots are first excised from the silver stained gel and transferred to a proteomic grade tube. Silver stained gel pieces are washed with SilverOUT[™] to remove inhibitory silver ions. The protein is then alkylated and reduced within the gel piece using the supplied aliquots of DTT and iodoacetamide. The proteins are then digested within the gel using our Mass Spectrometry Grade Trypsin and proprietary Digestion Buffer.

The digested peptides are extracted with Pep-Extract[™], a high diffusion peptide extraction buffer. The extracted peptides are suitable for mass spectrometry analysis without any subsequent treatments or cleaning procedures.

- InGel[™] Silver is supplied with:
- SilverOUT[™]: For removal of silver ions
 OneQuant[™] DTT: Reducing agent in single use aliquots to prevent contamination
- OneQuant[™] Iodoacetamide: Alkylating agent in single use aliquots to prevent contamination
- Trypsin, Mass Spectrometry Grade: Highly pure, autolysis resistant trypsin
- Trypsin Digestion Buffer: For optimal trypsin activity
- Pep-Extract[™]: For high level peptide extraction

FEATURES

- For the in-gel tryptic digestion of proteins
- Compatible with silver stained proteins
- Supplied with Mass spectrometry grade trypsin
- Supplied with destaining, reducing, alklating and peptide extraction reagents

APPLICATIONS

For MALDI peptide mass mapping and for LC MS/MS

CITED REFERENCES

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- Priyanka, A. et al (2016) Crystal structure of the Nterminal domain of human SIRT7 reveals a three helical domain architecture. Proteins.DOI: 10.1002/prot.25085
- Kanaujia, P.K. et al (2015) Proteomic analysis of Yersinia enterocolitica biovar 1A under iron-rich and iron-poor conditions indicate existence of efficiently regulated mechanisms of iron homeostasis J Proteomics. 124:39

Cat. No.	Description	Size
<u>786-241</u>	<u>InGel[™] Silver</u>	100 preps



In gel digestion of proteins in Coomassie and fluorescent stained gels

Provides a complete set of reagents for the ingel tryptic digestion and extraction of peptides for mass spectrometry (MALDI and LC MS/MS). The kit is specifically designed for use with Coomassie or fluorescent stained protein spots/bands.

The protein spots are first excised from the Coomassie or fluorescent stained gel. Stained gel pieces are washed with BlueOUT[™] to remove inhibitory stains. The protein is then alkylated and reduced within the gel piece using the supplied aliquots of DTT and iodoacetamide. The proteins are digested within the gel using our Mass Spectrometry Grade Trypsin and proprietary Digestion Buffer.

The digested peptides are extracted with Pep-Extract[™], a high diffusion peptide extraction buffer. The extracted peptides are suitable for mass spectrometry analysis without any subsequent treatments or cleaning procedures.

InGel[™] Blue is supplied with:

- BlueOUT[™]: For removal of Coomassie or fluroescent stains
- OneQuant[™] DTT: Reducing agent in single use aliquots to prevent contamination
- OneQuant[™] lodoacetamide: Alkylating agent in single use aliquots to prevent contamination
- Trypsin, Mass Spectrometry Grade
- Trypsin Digestion Buffer: For optimal trypsin activity
- Pep-Extract[™]: For high level peptide extraction

FEATURES

- For the in-gel tryptic digestion of proteins
- · Compatible with Coomassie and fluorescent stained proteins
- · Supplied with Mass spectrometry grade trypsin
- Supplied with destaining, reducing, alklating and peptide extraction reagents

CITED REFERENCES

 Ahmed, S. et al (2017) Stabilization of a soluble, native-like trimeric form of an efficiently cleaved Indian HIV-1 clade C envelope glycoprotein. J Biol Chem.doi: 10.1074/jbc.M117.776419

Cat. No.	Description	Size
<u>786-681</u>	<u>InGel[≞] Blue</u>	100 preps

InGel[™] Array

High throughput in gel digestion of protein spots

96-well format kit to process larger numbers of protein spots concurrently and is compatible with spot-picking instruments.

The protein spots are first excised from the silver stained gel and transferred to a proteomic grade titer plate. Silver stained gel pieces are washed with SilverOUT[™] to remove inhibitory silver ions. The proteins are then digested within the gel using a Mass Spectrometry Grade Trypsin and supplied Digestion Buffer.

The digested peptides are extracted with Pep-Extract[™], a high diffusion peptide extraction buffer. The extracted peptides are suitable for mass spectrometry analysis without any subsequent treatments or cleaning procedures.

InGel[™] Array is supplied with:

- SilverOUT[™]: For removal of silver ions
- Trypsin Digestion Buffer: For optimal trypsin activity
- Pep-Extract[™]: For high level peptide extraction
- InGel[™] Array titer plates and caps

Mass Spectrometry Grade Trypsin is available separately.

Cat. No.	Description	Size
<u>786-241A</u>	InGel [™] Array	500 preps



Figure 28: InGel™

silver scheme.

PROTEASE REMOVAL

Immobilized Soybean Trypsin Inhibitor

Immobilized Soybean Trypsin Inhibitor (STI) resin is designed for the efficient removal of trypsin, chymotrypsin and elastase proteases from protein digests. The action of the Immobilized STI resin will stop enzymatic reactions, in addition to removing the proteases and simplifying the analysis of the digested peptides.

The resin consists of the 20kDa Soybean Trypsin Inhibitor covalently coupled to agarose resin. The resin can be reused up to 10 times without significant loss in activity.

FEATURES

- Binding Capacity: >6mg trypsin/ml resin
- Support: 4% Agarose
- Ligand: Soybean Trypsin Inhibitor

APPLICATIONS

- Eliminating trypsin from protein digests
- Purification of trypsin, elastase and chymotrypsin



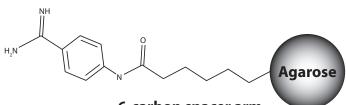
ρ-Aminobenzamidine Agarose

 ρ -Aminobenzamidine Agarose primary application is for the removal and/or purification of trypsin-like proteases. ρ -aminobenzamidine (PAB) is a synthetic inhibitor of trypsin-like proteases and has been covalently coupled to 6% cross-linked agarose.

ρ-aminobenzamidine agaroses have been used to purify a large range of specific proteins, including serine proteases.

For recombinant protein purification, the ρ -Aminobenzamidine Agarose can be used to remove the serine proteases (thrombin and enterokinase) that are used for cleavage of recombinant protein purification tags.

The p-Aminobenzamidine Agarose also contains a 6- carbon spacer arm between the p-Aminobenzamidine group and the agarose beads, making it suitable for coupling of small proteins and peptides. The long spacer arm minimizes steric hindrance allowing high efficient binding of ligands, including small proteins and peptides.



6-carbon spacer arm

Figure 23: p-Aminobenzamidine Agarose structure

FEATURES

- 90µm mean particle size
- 45-165µm particle size range
- Spherical, highly cross-linked 6% agarose
- 8-14mg trypsin/ml drained resin binding capacity
- 8μmol ρ-aminobenzamidine/ml drained resin ligand density
- 3-13 pH stability

APPLICATIONS

- Removal and/or purification of trypsin, trypsin-like serine proteases.
- Removal and/or purification of zymogens, inclduing urokinase and prekallikrein.
- Removal of thrombin and factor Xa have cleavage of tags from recombinant proteins

Cat. No.	Description	Size
<u>786-692</u>	p-Aminobenzamidine Agarose	25ml

A wide selection of protein extraction and lysis buffer systems are offered. The range includes products that maintain biological activity of proteins (PE LB[™] systems), strong chaotropic extraction buffers that are 2D compatible (2D-Xtract[™], FOCUS[™] Extraction Buffers) and extraction systems for total proteomes (FOCUS[™] Proteome kits).

Common lysis buffers (RIPA), extraction tools (grinding resins), enzymes (lysozyme and Zymolyase®), protease and phosphatase inhibitors and other extraction accessories are also offered.

PROTEIN EXTRACTION & LYSIS BUFFER (PE LB[™]) SYSTEMS

Lysis and extraction of biologically active proteins from cellular and tissue samples is the first critical step for biochemical analysis. The correct selection of lysis and extraction buffers requires knowledge of the proteins of interest and the stability of their biological activities.

The Protein Extraction & Lysis Buffer (PE LB[™]) systems ensure good protein recovery, while maintaining the biological activity of the proteins. The solubilized proteins are suitable for enzyme assays, electrophoresis, folding studies, chromatographic studies and many other downstream applications.

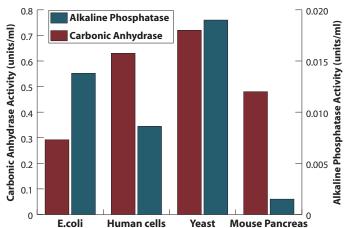


Figure 24: PE $LB^{\mathbb{M}}$ System maintains the biological activity of proteins. Extraction of carbonic anhydrase or alkaline phosphatase from E.coli, human cells, yeast and mouse pancreas with Bacterial, Mammalian Cell, Yeast and Tissue PE $LB^{\mathbb{M}}$ respectively. The resulting lysates were submitted to enzyme assays and both enzymes retain their biological activity.

The PE LB[™] systems offer a wide selection of buffers for lysis and extraction of proteins from bacteria, yeast, animal cells and tissues. The PE LB[™] systems are based on a proprietary combination of organic buffering agents, mild non-ionic detergents, and a combination of various salts to enhance extraction of proteins and maintain stability of biological activities of the proteins.

Depending on the application, additional agents such as chelating agents, reducing agents and protease and phosphatase inhibitors may be added to the PE $LB^{\mathbb{M}}$ buffer system.

The PE LB[™] systems are compatible with most downstream applications including enzyme assays, ELISA, chromatographic applications, gel electrophoresis, Western blotting and protein folding procedures.

Yeast PE LB[™]

Developed for the extraction of biologically active, soluble proteins from yeast cells. Yeast PE LB[™] is a proprietary improvement on the lyticase (Zymolyase®) based spheroplast preparation and extraction of soluble proteins from yeast cell method. Based on organic buffering agents and utilizes a mild non-ionic detergent, chelating agent, and a proprietary combination of various salts and agents to enhance extraction and stability of proteins.

A ready-to-use Zymolyase® preparation is also provided. Depending on the required downstream application, additional agents such as reducing agents and protease inhibitors may be added into Yeast PE LB[™]. Yeast PE LB[™] has been tested on several widely used yeast strains. Suitable for processing 100 x 50µl yeast cell pellets. Yeast PE LB[™] buffer is also available separately.

FEATURES

- · Eliminates the need for glass bead lysis
- Supplied as a kit, containing Zymolyase®

APPLICATIONS

- · Lysis and extraction of proteins from yeast cells
- Isolation of spheroplasts

CITED REFERENCES

Barajas, D. et al (2017) Generation of infectious recombinant Adeno-associated virus in Saccharomyces cerevisiae. PLoS One. https://doi.org/10.1371/journal.pone.0173010

Cat. No.	Description	Size
<u>786-178</u>	Yeast PE LB [™] Kit including Zymolyase [®]	100 preps
<u>786-179</u>	Yeast PE LB [™] , buffer only	500ml

Mammalian Cell PE LB[™]

Mammalian Cell PE LB[™] has been developed for extraction of total biologically active, soluble proteins from mammalian cultured cells. The Mammalian Cell PE LB[™] is based on organic buffering agents and utilizes a mild non-ionic detergent, chelating agent, and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. Depending on the required downstream application, additional agents such as reducing agents, phosphatase and protease inhibitors may be added into Mammalian Cell PE LB[™]. Mammalian Cell PE LB[™] has been tested on a wide variety of mammalian cells and can be used for both suspension and adherent cells.

FEATURES

 Compatible with most enzyme assays including reporter gene assays (β-galactosidase, luciferase, chloramphenicol acetyltransferase), kinases (protein kinase C, protein kinase A, tyrosine kinase) & immunoassays (ELISA, Western blots, RIA)

APPLICATIONS

- For extraction of soluble proteins from adherent and suspension animal cultured cells
- Suitable for most applications including enzyme and protein purification applications, electrophoresis, Western blotting and 2D-gel analysis

CITED REFERENCES

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- Battula, V.L. et al (2017) AML-induced osteogenic differentiation in mesenchymal stromal cells supports leukemia growth. JCl Insight https://doi.org/10.1172/jci.insight.90036.
- Ma, S.Y. et al (2017) Fucoxanthin inhibits profibrotic protein expression in vitro and attenuates bleomycin-induced lung fibrosis in vivo. Eur J Pharmacol.https://doi.org/10.1016/j. ejphar.2017.06.022
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More ciatations available at www.gbiosciences.com

Cat. No.	Description	Size
<u>786-180</u>	<u>Mammalian Cell PE LB</u> [™]	500ml

Protein Extraction & Lysis

Bacterial PE LB[™]

Extraction of bacterial and recombinant proteins

For the extraction of biologically active soluble proteins, including recombinant proteins, and inclusion bodies from bacterial cells. A proprietary improvement on the lysozyme based lysis method, which allows for the extraction of soluble proteins and concurrent removal of nucleic acids (DNA & RNA) released during cell lysis. The Bacterial PE LB[™] lysis eliminates viscosity build-up, allowing effective clarification with lower centrifugal forces.

Based on organic buffering agents and utilizes a mild non-ionic detergent, chelating agent, and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. Depending on the required downstream application, additional agents such as reducing agents and protease inhibitors may be added. Bacterial PE LB[™] has been tested for use with several widely used bacterial strains.

Supplied as a kit, which includes PE LB[™] Lysozyme, a modified lysozyme preparation that contains nucleases and results in optimal lysis and minimal contamination. Bacterial PE LB[™] buffer is also available separately for further downstream applications.

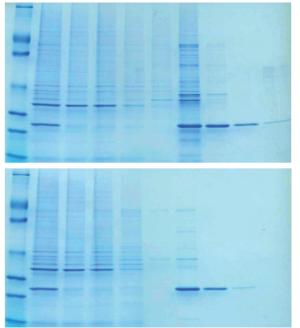


Figure 25: Bacteria expressing a His-tagged protein were lysed with Bacterial PE-LB[™] and the recombinant protein was purified with HOOK[™] 6X His Protein Purification kits (Top: Nickel resin; Bottom: Cobalt resin). Lane 1: PAGEmark[™] protein ladder; 2: Cleared lysate; 3: Flow through; 4-6: Washes; 7-9: Elutions.

FEATURES

- · Eliminates mechanical lysis and viscosity build-up
- Suitable for processing 100 x 50µl bacterial cell pellets

APPLICATIONS

- Lysis and extraction of proteins from bacterial cells
- · For the isolation of biologically active proteins

CITED REFERENCES

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- Miner-Williams, W. et al (2009) J. Agric. Food Chem. 57:2072 Bao, N. and Lu, C. (2008) Prin. Bacter. Detect.817 8

Cat. No.	Description	Size
<u>786-176</u>	Bacterial PE LB [™] Kit including PE LB [™] Lysozyme	100 preps
<u>786-187</u>	Bacterial PE LB [™] Kit including PE LB [™] Lysozyme	250 preps
<u>786-188</u>	Bacterial PE LB [™] Kit including PE LB [™] Lysozyme	500 preps
<u>786-177</u>	Bacterial PE LB [™] buffer only	500ml
<u>786-185</u>	Bacterial PE LB [™] buffer only	100ml
<u>786-186</u>	Bacterial PE LB [™] buffer only	250ml
<u>786-189</u>	Bacterial PE LB [™] 2X buffer only	250ml
<u>786-191</u>	Bacterial PE LB [™] in Phosphate Buffer	500ml

Tissue PE LB[™]

Developed for extraction of total biologically active, soluble proteins from animal tissues. Tissue PE LB[™] is based on an organic buffer and utilizes a mild non-ionic detergent, chelating agent, and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. Depending on the required downstream application, additional agents such as reducing agents and protease inhibitors may be added. Suitable for a wide variety of fresh and frozen animal tissues.

FEATURES

· Compatible with most enzyme assays including reporter gene assays (β-galactosidase, luciferase, chloramphenicol acetyltransferase), kinases (protein kinase C, protein kinase A, tyrosine kinase) & immunoassays (ELISA, Western blots, RIA)

APPLICATIONS

- · Soluble protein extraction from fresh and frozen animal tissue
- · Suitable for most applications including enzyme and protein purification applications, electrophoresis, Western blotting and 2D-gel analysis

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- More citations available at www.gbiosciences.com

Cat. No.	Description	Size
<u>786-181T</u>	<u>Tissue PE LB</u> [™] _	50ml
<u>786-181</u>	<u>Tissue PE LB</u> [™] _	500ml

Insect PE LB[™]

Insect PE LB[™] has been developed for extraction of total biologically active, soluble proteins from adherent or suspension cultured insect cells, including Sf9 and Sf21. Insect PE LB™ utilizes a mild non-ionic detergent and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. The Insect PE LB[™] is fully compatible with downstream processes, such as electrophoresis and chromatography. Depending on the required downstream application, additional agents such as reducing agents and protease inhibitors may be added into Insect PE LB[™].

FEATURES

- · Provides a simple and versatile method for protein extraction from adherent or suspended Sf9 and Sf21 insect cells
- Compatible with electrophoresis and chromatographic applications

APPLICATIONS

- For extraction of soluble proteins from cultured insect cells
- Suitable for most applications including enzyme and protein purification applications, electrophoresis, Western blotting and 2D-gel analysis

Cat. No.	Description	Size
<u>786-411</u>	Insect PE LB [™]	250ml

Total Protein Extraction (TPE[™])

For the extraction of total protein from cells & tissues for SDS-PAGE analysis

Universal lysis system for the solubilization of total proteins from animal, plant, yeast, bacteria, and other biological samples. Samples are ground in the buffer provided and then heated to solubilize the total protein.

The TPE[™] kit provides a two component protocol that eliminates clump formation, protein loss, and other problems associated with total protein extraction procedures.

The TPE[™] kit is based on optimized concentration of Tris and SDS and is suitable for running denaturing electrophoresis and other downstream applications.

FEATURES

- · Ready-to-use buffers for extraction of total protein
- Two component extraction protocol
- Based on an optimized concentration of Tris and SDS
- Supplied with sufficient reagents for 50 x 250mg preparations

APPLICATIONS

· Solubilization of total proteins for electrophoresis & more

CITED REFERENCES

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- Prathyumnan, S. et al Int. J. Cur. Sci. Res. 3:120 4.

Cat. No.	Description	Size
<u>786-225</u>	Total Protein Extraction (TPE [™]) Kit	50 preps

IBS[™] Buffer

Inclusion bodies solubilization buffer

The IBS[™] buffer is specifically developed for solubilization of inclusion bodies.

Simple to use protocol as inclusion bodies are suspended in IBS™ Buffer, where they readily dissolve releasing the proteins of interest. Once the inclusion bodies are solubilized, the sample is ready for further analysis and other downstream applications. Supplied with optional DTT.

We offer IBS-HP[™] Buffer for the solubilization of inclusion bodies containing highly hydrophobic proteins.

CITED REFERENCES

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- Hossain, A. et al (2004) Mol Endocrinol 18:1428 3. Edward, K. et al (2004) J Cell Sci 117:5875

Cat. No.	Description	Size
<u>786-183</u>	IBS [™] Buffer Kit	100ml
<u>786-183HP</u>	IBS-HP [™] Buffer Kit	100ml

RIPA Lysis & Extraction Buffer

A complete lysis buffer for the release of cytoplasmic, membrane and nuclear proteins from adherent and suspension cultured mammalian cells. The RIPA lysis buffer is fully compatible with many applications, including reporter assays, protein assays, immunoassays and other protein purification techniques.

RIPA Lysis Buffer does not contain protease inhibitors, however it is fully compatible with our range of individual protease inhibitors and cocktails.

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- More citations available at www.gbiosciences.com

Cat. No.	Description	Size
<u>786-489</u>	RIPA Lysis & Extraction Buffer	100ml
<u>786-490</u>	RIPA Lysis & Extraction Buffer	500ml

Total Proteome Extraction Kits

FOCUS[™] PROTEOME KITS

Isolate total proteomes from various species

An effective proteome analysis requires the preparation of a sample to bring the wide range of protein species into the dynamic range of detection. The absence of any standardized procedures for sample preparation has made proteome analysis extremely complicated, requiring a multitude of complicated skills, expensive equipment, and resources.

FOCUS[™] Proteome Kits are for the preparation of total protein, including soluble, insoluble, membrane, cytoplasmic, nuclear, signal, phospho- and glyco-proteins. The FOCUS[™] Proteome Kits are suitable for biological samples from tissues, cells, plants, yeast, bacteria and insects. These kits are simple to use, save time, improve the quality of protein analysis and enhance the chances of discovery of novel proteins. The kits are suitable for the analysis of proteins using electrophoresis and other biochemical techniques.

FOCUS[™] Mammalian Proteome

Extracts and solubilizes nearly all of the proteins from mammalian samples, including membrane as well as soluble proteins, by a strong chaotropic extraction buffer to solubilize even the most difficult proteins. Suitable for biological samples from animal tissues and adherent and suspension cells.

FEATURES

- Single step extraction protocol
- · Supplied with a strong, chaotropic extraction buffer
- + For 50 x 100mg animal tissue or 50 μl wet cell pellets

APPLICATIONS

- · Extraction of total proteins from mammalian tissues and cells
- Sample preparation for 2D gel electrophoresis

CITED REFERENCES

 Kan, F. et al (2017) Proteomic and transcriptomic studies of HBV-associated liver fibrosis of an AAV-HBV-infected mouse model. BMC Genomics. https://doi.org/10.1186/s12864-017-3984-z

Cat. No. Description Size

<u>786-246</u> <u>FOCUS[™] Mammalian Proteome</u> 50 preps

FOCUS[™] Bacterial Proteome

Specifically designed for bacterial research and supplied with bacteria specific reagents. Extracts and solubilizes nearly all of the proteins from E.coli, including membrane as well as soluble proteins. Extraction is based on the gentle lysis of bacterial cells with LongLife[™] Lysozyme enzyme, followed by extraction of total proteome with the supplied strong chaotropic extraction buffer that solubilizes even the most difficult proteins. Our LongLife[™] Lysozyme has improved stability and shelf life.

FEATURES

- Simple extraction protocol
- Supplied with bacterial specific lytic enzyme preparation
- · Supplied with a strong, chaotropic extraction buffer
- Suitable for extraction from 50 x 50µl bacterial cell pellet

APPLICATIONS

 Suitable for sample preparation for 2D gel electrophoresis and other applications

CITED REFERENCES

- Raza, W. et al (2016) Volatile organic compounds produced by Pseudomonas fluorescens WR-1 restrict the growth and virulence traits of Ralstonia solanacearum. Microbiol. Res. 192:103
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Cat. No. Description Size 786-258 FOCUS[™] Bacterial Proteome 50 preps

FOCUS[™] Insect Proteome

Extracts and solubilizes nearly all of the proteins from insect cell cultures (i.e. Sf9 and Sf21), including membrane as well as soluble proteins, using a strong chaotropic extraction buffer to solubilize even the most difficult proteins.

FEATURES

- Single step extraction protocol
- For the extraction of protein from 50 x 50µl insect cell pellets

APPLICATIONS

· For 2D gel electrophoresis and other applications

Cat. No.	Description	Size
<u>786-360</u>	FOCUS [™] Insect Proteome	50 preps

FOCUS[™] Yeast Proteome

Specifically designed for yeast research and supplied with yeast specific reagents. Extracts and solubilizes nearly all of the proteins from yeast, including membrane and soluble proteins. Extraction is based on gentle lysis of yeast cells with a yeast lytic enzyme preparation, LongLife[™] Zymolyase[®], which has improved stability and shelf life. Enzymatic action is followed by extraction of total proteome with the supplied strong chaotropic extraction buffer that solubilizes even the most difficult proteins.

FEATURES

- Supplied with yeast specific lytic enzyme preparation and a strong proprietary chaotropic extraction buffer
- Suitable for 50 x 60µl yeast cell pellet preparations

APPLICATIONS

 Suitable for sample preparation for 2D gel electrophoresis and other applications

Cat. No.	Description	Size
<u>786-257</u>	FOCUS [™] Yeast Proteome	50 preps

FOCUS[™] Plant Proteome

Specifically designed for plant research and supplied with plant specific reagents, including reagents for removal of plant pigments and other natural products that may interfere with protein analysis. Extracts and solubilizes nearly all of the proteins from plants, including membrane as well as soluble proteins. Supplied with a strong proprietary chaotropic extraction buffer to solubilize even the most difficult proteins.

FEATURES

- Simple extraction protocol
- Supplied with reagents for removal of plant pigments and other natural products that may interfere with protein analysis
- · Supplied with a proprietary chaotropic extraction buffer
- Extracts plant proteome from 25 x 0.5gm plant tissue preparations

APPLICATIONS

 Suitable for sample preparation for 2D gel electrophoresis and other applications

CITED REFERENCES

1. Dagda, R.K. et al (2014) J. Neurochem. 128:864

Cat. No.	Description	Size
<u>786-259</u>	FOCUS [™] Plant Proteome	25 preps

DENATURING EXTRACTION BUFFERS

FOCUS™ Extraction Buffers

Chaotropic extraction buffers that preserve the native charge of proteins

One of the most important considerations before running 2D gel electrophoresis is the choice of protein solubilization buffers. The suitable buffer must solubilize proteins effectively, without disturbing the native charge of the proteins. Urea, a common chaotrope, is widely used for solubilization and denaturation of proteins. One of the disadvantages of using urea is carbamylation. Urea in water exists in equilibrium with ammonium cyanate, the level of which increases with increasing temperature and pH. Cyanate reacts with α -amino and ϵ -amino groups of proteins and induces a change in the isoelectric point of proteins. This leads to artifactual results and therefore carbamylation must be avoided.

One way to minimize the risk of carbamylation is to prepare the urea based reagents fresh before each use. G-Biosciences has developed a series of dry urea based pre-mixed and ready-touse solubilization buffers. Simply add an appropriate volume of the supplied rehydration buffer to the dry buffer and then use to solubilize proteins, saving time and improving the quality of IEF/2D gel electrophoresis.

 $\mathsf{FOCUS}^{\scriptscriptstyle \rm M}$ Extraction Buffers are also designed to be used as rehydration buffers for IPG strips.

FOCUS[™] Extraction Buffers are experimentally optimized concentrations of critical agents, buffering and stabilizing agents, including urea, thiourea, Nonidet® P-40, CHAPS, and sulfobetaines (SB). The FOCUS[™] Extraction Buffers are designed to produce optimal protein extraction and improved spot resolution for 2D gel analysis.

A range of FOCUS[™] Extraction Buffers have been developed and depending on the nature of the samples, one or more of the buffers suitable for your applications can be ordered. FOCUS[™] Extraction Buffer-I is suitable for most applications, however for stronger solubilization effects, we recommend FOCUS[™] Extraction Buffer-II, -III, -IV, -V or -VI.

For analysis of a proteome, a single buffer may not be suitable and sequential solubilization using different FOCUS[™] Extraction Buffers will help in identifying new proteins.

FEATURES

- · Convenient and simple to use extraction buffers
- · Simply hydrate and use
- Prevents urea induced protein carbamylation
- · Prevents waste of unused reagents

APPLICATIONS

• Suitable for sample extraction and solubilization for 2D gel electrophoresis and other application

CITED REFERENCES

 Wang, T. et al (2007) Biochem. Bioph. Res. Co. 352:203

 Kim, Y.H. et al (2007) Life Sciences. 81:1167

Cat. No.	Description	Size
<u>786-220</u>	FOCUS [™] Extraction Buffer I	For 50ml
<u>786-221</u>	FOCUS [™] Extraction Buffer II	For 50ml
<u>786-222</u>	FOCUS [™] Extraction Buffer III	For 50ml
<u>786-223</u>	FOCUS [™] Extraction Buffer IV	For 50ml
<u>786-219</u>	FOCUS [™] Extraction Buffer V	For 50ml
<u>786-233</u>	FOCUS [™] Extraction Buffer VI	For 50ml
786-234	FOCUS [™] Extraction Buffers I-VI Trial kit	For 10ml each buffer

Protein Extraction Accessories

PROTEIN EXTRACTION & ISOLATION ACCESSORIES

EZ-Grind



Figure 26: EZ-Grind[™] showing pestles and prepacked grinding resin.

A highly efficient grinding resin that is pre-aliquoted into 1.5ml grinding tubes and is supplied with matching pestles. The resin is designed for optimal grinding of biological samples for the extraction of both proteins and DNA. The resin is a neutral abrasive material that does not bind proteins or nucleic acids.

Supplied with 20 resin tubes and 20 matching pestles.

FEATURES

- Disrupts small tissue and cell samples for protein extraction
- Uses 1.5 ml microcentrifuge tubes, grinding resin, and disposable pestles

Process up to 100mg of sample per tube in about 10 min

CITED REFERENCES

- Barter, M.J. et al (2015) Stem Cell Epigenet. DOI: 10.1002/stem.2093
- 2. Avasarala, S. et al (2013) PLOS. DOI: 10.1371/journal.pone.0057285 3. lordanskiy, S. et al (2010) Retrovirology. 7:85



Molecular Grinding Resin[™]

Available with matching pestles & tubes

Molecular Grinding Resin[™] is ideal for grinding small samples and the subsequent preparation of proteins and nucleic acids. The resin consists of high tensile micro particles, which effectively disrupt nuclei and other cellular organelles. The resin is fully compatible with any homogenization technique, including high speed mechanical grinders and sonicators.

Molecular Grinding Resin[™] does not bind proteins or nucleic acids, minimizing loss. Simply mix the Molecular Grinding Resin[™] with the biological samples and grind or homogenize the sample.

Supplied with enough resin for 200 isolations from 100mg tissue.

CITED REFERENCES

- Lajnaf, R. et al (2017) The foaming properties of camel and bovine whey: the impact of pH and
- heat treatment. Food chemistry. https://doi.org/10.1016/j.foodchem.2017.07.064 Lajnaf, R. et al (2017) The effect of pH and heat treatments on the foaming properties of purified α-lactalbumin from camel milk. Colloids and Surfaces B. https://doi.org/10.1016/j. colsurfb.2017.05.002

More citations available at www.gbiosciences.com

Cat. No.	Description	Size
<u>786-138</u>	Molecular Grinding Resin [™]	5 x 0.5ml resin
786-138PR	Molecular Grinding Resin [™] _ with pestles & tubes	5 x 0.5ml resin & 100 pestles & tubes
<u>786-138P</u>	Pestles & Tubes	100 pestles & tubes

LongLife[™] Enzyme Preparations

Enzymes regularly used in laboratory applications often require preparation of fresh solution before each use. Making fresh enzyme solution for each application is time consuming and wasteful. A wide variety of enzyme preparations in a ready-to-use format are offered.

LongLife[™] enzyme preparations have a long shelf life and no weighing or buffer preparation is needed; simply take an aliquot and add to your sample. LongLife[™] enzyme preparations contain cofactors necessary for optimal enzymatic activity. Supplied in suspension form and when stored properly have a one year shelf life.

ENZYMES OFFERED

- LongLife[™] Zymolyase®: digestion of yeast & fungal cell walls
- LongLife[™] Lysozyme: digestion of bacterial cell walls
- LongLife[™] PE LB Lysozyme: digestion of bacterial cell walls & nucleic acids. Fully compatible with PE LB buffer system. Reduces viscosity build-up due to presence of nucleases.
- LongLife[™] Proteinase K: digestion of proteins in nucleic acid preparations
- LongLife[™] Nuclease: removal of nucleic acids
- LongLife[™] RNase: digestion of RNA
- LongLife[™] DNase: digestion of DNA

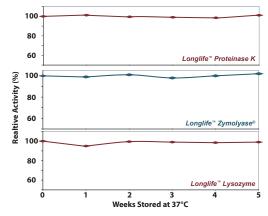


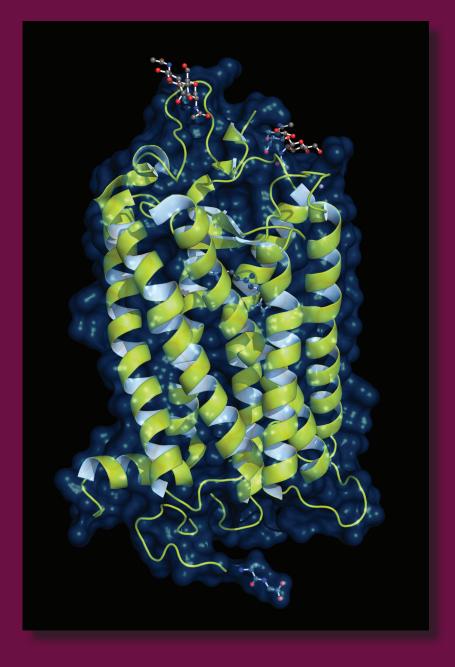
Figure 27: LongLife[™] Enzymes are highly stable. Each enzyme preparation was tested over a period of 4 weeks at 37°C: and compared with LongLife[™] enzyme preparations stored at -20 °C.

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- pv. tomato DC3000.J Bacteriol.doi : 10.1128/JB.00276-16 2. Rodriguez, M. et al (2014) J. Biomech. Eng. doi:10.1115/1.4027145
 - З. Xiao, S. et al (2014) J. Peptide Sci. 20:216
 - Lam, H. N. et al (2014) PLOS. DOI: 10.1371/journal.pone.0106115 Park, S.H. et al (2013) Microbiology. 159: 296 4.
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 - 6. Lynch, J.B. and Sonnenburg, J.L. (2012) Mol. Microbiol. 85:478
 - 7. Rodriguez, A. et al (2011) Biophys. J. 101:2455
 - 8. Butcher, B.G. et al (2011) J Bacteriol 193:4598 Markel, E. et al (2011) J Bacteriol 193:5775 9.
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 - Robert, A.W. et al (2016) Tissue-Derived Signals for Mesenchymal Stem Cell Stimulation: Role of 11. Cardiac and Umbilical Cord Microenvironments. Cells Tissues Organs. DOI:10.1159/000450600
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 - 14. Whitaker, V.M. et al (2007) J Amer Soc Hort Sci 132:534 15
 - Funk, I. et al (2017) Production of dodecanedioic acid via biotransformation of low cost plant-oil derivatives using Candida tropicalis. J Ind Microbiol Biotechnol. https://doi.org/10.1007/ s10295-017-1972-6

Cat. No.	Description	Size
<u>786-036</u>	<u>LongLife[™] Zymolyase® [1.5U/µl]</u>	2 x 0.5ml
<u>786-037</u>	<u>LongLife[™] Lysozyme [1,500U/µl]</u>	2 x 0.5ml
<u>786-042</u>	<u>LongLife[™] PE LB Lysozyme [1,500U/µl]</u>	2 x 0.5ml
<u>786-038</u>	LongLife [™] Proteinase K [5mg/ml]	2 x 0.5ml
<u>786-039</u>	<u>LongLife[™] Nuclease [10U/µl]</u>	2 x 0.5ml
<u>786-040</u>	<u>LongLife[™] RNase [10U/µl]</u>	2 x 0.5ml
<u>786-041</u>	<u>LongLife[™] DNase</u>	0.5ml







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