G-Biosciences

Protease & Phosphatase Inhibitors & Proteases

A handbook & selection guide for inhibitors of protease & phosphatases & for proteases & assays

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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.

G-Biosciences offers a selection of specific proteases that are designed for use in peptide fragmentation for mass spectrometry analysis or protein sequencing.

PROTEASE INHIBITOR COCKTAILS

ProteaseArrest™ Cat. # 786-108, 786-329

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 (Ca^{2+}, Mg^{2+} or Mn^{2+}) for their biological activity. In addition, EDTA will inhibit the purification of proteins using immobilized metal affinity chromatography (IMAC).

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 offered as a dry format, as ProteCEASE™, for those who prefer reconstitution prior to use.

ProteaseArrest™ Outperforms Tablet Cocktails

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 cocktails demonstrate greater inhibition levels compared to similar protease inhibitor cocktails, including tablet formats (see figure 1).

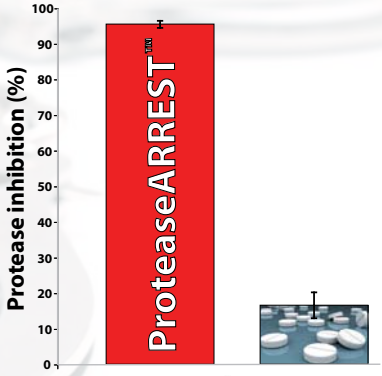
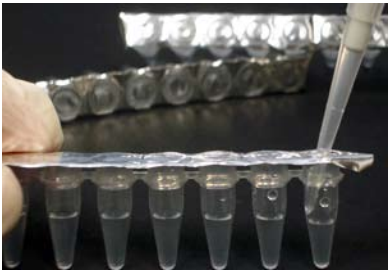


Figure 1: ProteaseArrest™ outperforms tablet format protease inhibitor cocktails. Protease inhibition in mouse pancreas lysate with ProteaseArrest (EDTA-free) and a commercially available EDTA-free tablet protease cocktail was compared, using Protease Screening Kit™. The assay used 0.5mg/ml pancreas lysate and incubation conditions of 37°C for 2.5 hours. ProteaseArrest™ inhibited over 95% of total proteases, almost 80% more protease inhibition compared to the tablet protease inhibitor cocktail.



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 (1).

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.

REFERENCES & CITATIONS

1. *Kitareewan, S. et al (2007) J Natl Cancer Inst. 99: 41.*
2. *Rocnik, J.L et al (2006) Blood. 108: 1339.*
3. *Yoshino, O. et al (2006) PNAS. 103: 10678.*
4. *Xie, H. et al (2006) Antimicrob. Agents Chemother. 50: 3070.*
5. *Gennidakis, S. et al (2007) Plant J. 52: 839.*
6. *Timney, B.L. et al (2006) J. Cell Biol. 175: 579.*

FOCUS™ ProteaseArrest™ Cat. # 786-108F

A 2D electrophoresis & mass spectrometry compatible protease inhibitor cocktail

FOCUS™ ProteaseArrest™ is 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™ Protease Arrest™ at 1X concentration in extraction buffer at pH 7-8 inhibits over 90% of protease activities.

FOCUS™ Protease Arrest™ 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 or 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.

REFERENCES & CITATIONS

1. *Zanello, S.B. et al (2006) Curr. Eye Research. 21: 825*

*Prote*CEASE™ Cat. # 786-326, 786-326T, 786-327, 786-328, 786-334, 786-335, 786-336

A protease inhibitor cocktail for large scale preparative applications

*Prote*CEASE™ is a dry format version of our ProteaseArrest™ for large scale preparative applications and for those who prefer reconstitution prior to use.

*Prote*CEASE™ 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 *Prote*CEASE™ will maintain activity of proteins dependent on divalent cations and will not inhibit the purification of proteins with immobilized metal affinity chromatography (IMAC).

*Prote*CEASE™ has been specifically developed for large scale preparative applications and is available in two vial sizes:

*Prote*CEASE™-50 for 50ml of lysis buffer

*Prote*CEASE™-100 for 100ml of lysis buffer.

*Prote*CEASE™-50 is available in packs of 10 or 20 vials for 500ml and 1 liter total volume and *Prote*CEASE™-100 is available in packs of 10 for 1 liter total volume.

Species Specific Protease Inhibitors

Bacterial ProteaseArrest™ Cat. # 786-330

A 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.

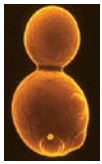
An optional EDTA solution is provided for enhanced metalloprotease inhibition. It is not present in the actual Bacterial ProteaseArrest™ cocktail as it would inhibit the activity of proteins that require divalent cations (Ca^{2+}, Mg^{2+} or Mn^{2+}) for their biological activity. In addition, EDTA will inhibit the purification of proteins using immobilized metal affinity chromatography (IMAC).

Plant ProteaseArrest™ Cat. # 786-332

A 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.

Yeast/ Fungal ProteaseArrest™

Cat. # 786-333



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

Mammalian ProteaseArrest™ Cat. # 786-331

A 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 (Ca^{2+}, Mg^{2+} or Mn^{2+}) for their biological activity. In addition, EDTA will inhibit the purification of proteins using immobilized metal affinity chromatography.

Recom ProteaseArrest™ Cat. # 786-376

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 (Ca^{2+}, Mg^{2+} or Mn^{2+}) 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.

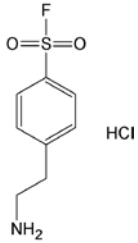
Individual Protease Inhibitors

A selection of individual protease inhibitors are offered for researchers who wish to design their own cocktails, supplement existing cocktails or screen for specific proteases.

The individual protease inhibitors are offered as a dry powder.

AEBSF Cat. # 786-053

4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride



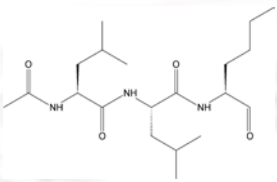
Specificity: Specific irreversible inhibitor of serine proteases, including chymotrypsin, kallikrein, plasmin, thrombin and trypsin. A stable non-toxic alternative to PMSF. A β-secretase inhibitor that inhibits β-amyloid peptide (Aβ) production and enhances amyloid precursor protein (sAPPUa) secretion in several cell lines at millimolar concentrations. Recommended concentrations for use are 0.1-1 mM. AEBSF has been used in cell culture in concentrations of up to 0.25 mM.

Solubility: Soluble in water (50 mg/mL stable for up to six months if stored refrigerated at a pH of less than 7. If a pH of greater than 7 is required, pH adjustment should be made just prior to use.)

Molecular weight: 239.7

ALLN Cat. # 786-057

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



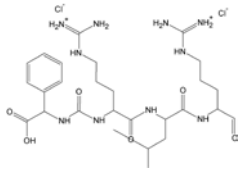
Specificity: Cell permeable peptide aldehyde inhibitor of calpain I (K_i = 190 nM) and to a lesser extent calpain II (K_i = 220 nM). Also inhibits other neutral cysteine proteases, cathepsin B (K_i = 150 nM), and cathepsin L (K_i = 500 pM). and the proteasome (K_i = 6 μM). Modulates the processing of the β-amyloid precursor protein (βAPP) to β-amyloid (Ab). Protects against neuronal damage caused by hypoxia and ischemia. Inhibits apoptosis in thymocytes and metamyelocytes. Also inhibits reovirus-induced apoptosis in L929 cells. Inhibits the proteolysis of IkB-a and IkB-b by the ubiquitin-proteasome complex. Inhibits cell cycle progression at G1/S and metaphase/anaphase in CHO cells by inhibiting cyclin B degradation. Also prevents nitric oxide production by activated macrophages by interfering with transcription of the inducible nitric oxide synthase gene.

Solubility: Soluble in DMSO or ethanol

Molecular weight: 383.5

Antipain, Dihydrochloride Cat. # 786-045

[(S)-1-Carboxy-2-Phenyl]-carbamoyl-Arg-Val-arginal



Specificity: Peptidyl arginine aldehyde protease inhibitor produced by actinomycetes. Inhibits Ca^{2+}-dependent endopeptidases, including papain, trypsin-like serine proteases, some cysteine proteases (IC50 = 300 µM) and to a lesser extent plasmin. Higher specificity for trypsin and papain compared to leupeptin. Effective concentrations for use are 1-100 µM.

Solubility: Soluble in water (50 mg/mL), methanol and DMSO (Stock solution: 10mM).

Molecular weight: 677.6

Aprotinin Cat. # 786-046

Also known as bovine pancreatic trypsin inhibitor.

Specificity: A broad range, competitive and reversible inhibitor of chymotrypsin, plasmin (K_d = 2.3 x 10_{-10} M), trypsin (K_d = 5 x 10^{-14} M), kallikrein (K_d = 1 x 10^{-7} M) and other serine proteases. Useful as a serine protease inhibitor during purification of proteins and in studies of zymogen activation systems. Effectively inhibits target proteases at equimolar concentration. pH optimum 5 - 7, pI 10.5. Aprotinin works by blocking the active sites of enzymes. Binding is reversible with most aprotinin-protease complexes dissociating at pH > 10 or < 3.

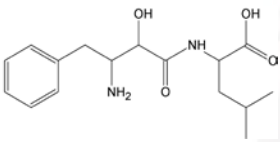
Solubility: Soluble in water (Stock solution: 10mM).

Molecular weight: 6512

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

Bestatin Cat. # 786-047

[(2S, 2R)-3-Amino-2-hydroxy-4-Phenylbutanoyl]-L-Leucine



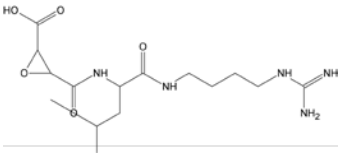
Specificity: Competitive inhibitor of surface aminopeptidases, including aminopeptidase B (K_i=2nM), leucine aminopeptidase (K_i=20nM). Also inhibits aminopeptidases N; does not inhibit endoproteases, aminopeptidase A, trypsin, chymotrypsin, elastase, papain, pepsin, or themolysin. Bestatin has been shown to ctivate macrophages and T lymphocytes as well as contain antitumor properties.

Solubility: Soluble up to 5mg/ml in methanol or 1mg/ml in 0.15M NaCl.

Molecular weight: 308.4

E-64 Cat. # 786-049

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



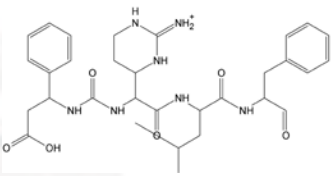
Specificity: Irreversible inhibitor of cysteine proteases; does not inhibit serine proteases. Interacts with the Sn subsites of proteases. Has no action on cysteine residues in other proteins. Inhibits activation-induced programmed cell death and restores defective immune responses in HIV+ donors. Specific active site titrant. The trans-epoxysuccinyl group (active moiety) of E-64 irreversibly binds to an active thiol group in many cysteine proteases, such as papain, actinidase, and cathepsins B, H, and L to form a thioether linkage. E-64 is a very useful cysteine protease inhibitor for use in in vivo studies because it has a specific inhibition, it is permeable in cells and tissues and has low toxicity. A suggested stock solution is a 1mM aqueous solution. The effective concentration for use as a protease inhibitor is 1 to10 µM.

Solubility: Soluble in DMSO (25mg/ml) and aqueous buffers (20mg/ml).

Molecular weight: 357.4

Chymostatin Cat. # 786-048

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



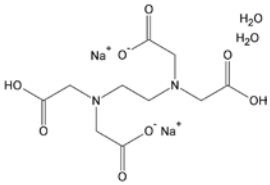
Specificity: Inhibits serine proteases having a chymotrypsin-like specificity, including α, β, γ, and δ chymotrypsin, and most cysteine proteases including cathepsins B, H, L. Chymostatin weakly inhibits human leucocyte elastase. It is often included in protease inhibitor cocktails used with plant extracts. It is effective at a final concentration of 100 to 200 µg/ml (10 to 100 µM).

Solubility: Soluble in DMSO (10 mM Stock solutions).

Molecular weight: 604.7

EDTA-Na$_2$ Cat. # 786-050

Ethylenediamine-tetraacetic acid disodium salt dihydrate



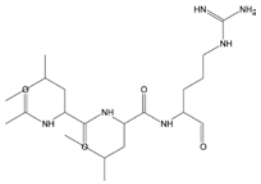
Specificity: Metal chelator that inhibits metalloproteases. EDTA-Na$_2$ is commonly used in cell culture control. It is clear, colorless, & odorless.

Solubility: Soluble in water (0.1 M at 20 °C).

Molecular weight: 372.24

Leupeptin Cat. # 786-051

Acetyl-Leu-Leu-Arg-al, N-Acetyl-L-leucyl-L-leucyl-L-argininal hemisulfate salt



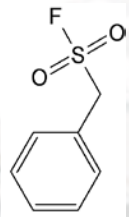
Specificity: Inhibits serine, plasmin, porcine kallikrein and cysteine proteases, including papain and cathepsin B. No inhibition found with pepsin, cathepsins A and D, thrombin, or α-chymotrypsin. Effective concentration 10-100 µM. Leupeptin is often used during in vitro experiments when a specific enzymatic reaction is being studied.

Solubility: Soluble in water (stable for 1 week at 4ºC and 1 month at -20ºC), ethanol, acetic acid and DMF (Stock solution: 10 mM).

Molecular weight: 426.6

PMSF Cat. # 786-055

Phenylmethanesulfonyl fluoride



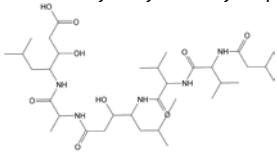
Specificity: Irreversible inhibitor of serine proteases, including trypsin and chymotrypsin. Also inhibits cysteine proteases and mammalian acetylcholinesterase. Not as effective or as toxic as DFP. Effective concentration 0.1-1 mM. Half-life = 1 hr. at pH 7.5.

Solubility: Soluble in dry solvents (methanol, ethanol and 2-propanol): 200 mM Stock solution are stable for months at 4°C.

Molecular weight: 174.2

Pepstatin Cat. # 786-052

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



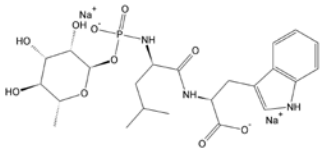
Specificity: A potent inhibitor of various aspartic proteases, including cathepsin D, renin, pepsin, bacterial aspartic proteases and HIV proteases.

Solubility: 10% acetic acid in methanol (1 mg/mL).

Molecular weight: 685.9

Phosphoramidon Cat. # 786-054

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



Specificity: Inhibits some metalloproteases, including thermolysin, collagenase and bacterial metallo proteases from Bacillus subtilis, Streptomyces griseus and Pseudomonas aeruginosa (metallo elastase). Strongly inhibits mammalian enkephalinase. Does not inhibit trypsin, papain, chymotrypsin or pepsin. Effective concentrations between 1-10 µM.

Solubility: Soluble in water, methanol and DMSO.

Molecular weight: 543.5

Protease Inhibitor Set Cat. # 786-207

100X Concentrated Protease Inhibitor Selection

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

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

- **AEBSF**
- **ALLN**
- **Antipain, dihydrochloride**
- **Aprotinin**
- **Bestatin**
- **Phosphoramidon**
- **E-64**
- **EDTA-Na$_2$**
- **Leupeptin**
- **Pepstatin**
- **Chymostatin**
- **PMSF**

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.

Protease Inhibitor Selection Guide & Ordering Information

Cat. #	Protease Inhibitor	Specificity	Solubility	Molecular Weight	Quantity Supplied
786-053	**AEBSF** 4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride	Specific irreversible inhibitor of serine proteases, including chymotrypsin, kallikrein, plasmin, thrombin and trypsin. A stable non-toxic alternative to PMSF.	H_2O	239.7	1g
786-057	**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
786-045	**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_2O, Methanol, DMSO	677.6	5mg
786-046	**Aprotinin** ***Also known as bovine pancreatic trypsin inhibitor.***	A broad range, competitive and reversible inhibitor of chymotrypsin, plasmin, trypsin, kallikrein and other serine proteases.	H_2O	6512	100mg
786-047	**Bestatin** [(2S, 2R)-3-Amino-2-hydroxy-4-Phenylbutanoyl]-L-Leucine	Competitive inhibitor of surface aminopeptidases, including aminopeptidase B (K_i=2nM), leucine aminopeptidase (K_i=20nM). Also inhibits aminopeptidases N; does not inhibit endoproteases.	Methanol (<5mg/ml), NaCl [0.15M] (<1mg/ml)	308.4	10mg
786-048	**Chymostatin** N-[(S)-1-carboxy-isopentyl)-carbamoyl-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
786-049	**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
786-050	**EDTA-Na_2** Ethylenediamine-tetraacetic acid disodium salt dihydrate	Metal chelator that inhibits metalloproteases.	H_2O	372.24	100g
786-051	**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_2O, Ethanol, Acetic Acid, DMF	426.6	25mg
786-052	**Pepstatin** Isovaleryl-Val-Val-AHMHA-Ala-AHMHA where AHMHA= (3S, 4S)-4-amino-3-hydroxy-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
786-054	**Phosphoramidon** N-alpha-L-rhamnopyranosyloxy (hydroxyphosphinyl)-L-Leucyl-L-Tryptophan	Inhibits some metalloproteases, including thermolysin, collagenase and bacterial metallo proteases from Bacillus subtilis, Streptomyces griseus and Pseudomonas aeruginosa (metallo elastase).	H_2O, Methanol, DMSO	543.5	10mg
786-055	**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

More information online... www.GBiosciences.com

Protease Assays & Screening Systems

*Prote*SEEKER™ Cat. # 786-325

Identify Destructive Proteases

*Prote*SEEKER™ identifies specific types of proteases with a panel of twelve protease inhibitors and a sensitive colorimetric protease screening assay. *Prote*SEEKER™ allows researchers 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, *Prote*SEEKER™ can be used to test existing protease inhibitor cocktails and identify their inadequacies and therefore supplement in additional protease inhibitors.

*Prote*SEEKER™ protease screening assay consists of a ready-to-use 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.

*Prote*SEEKER™ kit components include the following individual protease inhibitors as well as the Protease Screening Kit and is sufficient for 50 assays:

Protease Inhibitor	Inhibits
AEBSF	Serine proteases (trypsin, chymotrypsin, plasmin, plasma kallikrein, & thrombin
Antipain, dihydrochloride	Papain, trypsin & plasmin
Aprotinin	Serine proteases (plasmin, kallikrein, trypsin, chymotrypsin)
Bestatin	Amino-peptidases & other exopeptidases (not carboxypeptidases)
Phosphoramidon	Thermolysin, collagenase & other metalloendoproteinases
EDTA-Na_2	Metalloproteases
E-64	Papain & other cysteine proteases (cathepsin B & L)
Leupeptin	Serine & Cysteine proteases (Plasmin, Trypsin, Papain & Cathepsin B)
Pepstatin	Aspartic proteases (pepsin, renin, cathepsin D, chymosin)
Chymostatin	α, β, γ, δ chymotrypsin
PMSF	Serine proteases (chymotrypsin, trypsin & thrombin), cysteine proteases (papain)

Protease Screening Kit Cat. # 786-137

Does your sample contain protease activity?

The Protease Screening Kit provides you with 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 micro well format.

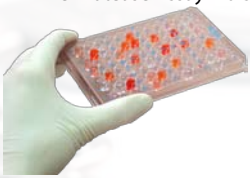
REFERENCES & CITATIONS

1. Person, M.D. et al (2006) J. Biomol. Tech. 17: 145.

2. Razeghi, P. et al (2007) Mol. Cell Cardiol. 42: 449.

Protease Assay Kit Cat. # 786-028

For Assay of Protease Activity



The Protease Assay Kit is designed for the quantitative determination of proteases present in a protein sample, using a dye-labeled protein substrate.

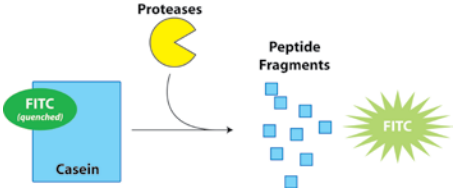
The proteases present in the sample of interest will digest the protein substrate and release dye labeled peptides. The absorbance of the dye-labeled peptide is measured at 570nm for determination of protease activity.

Chemically stabilized Trypsin (MSG-Trypsin™) is supplied with the kit as a general protease standard; however, other specific protease standards can also be used. MSG-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.

Fluoro™ Protease Assay Cat. 786-320

A Fluorometric, Quantitative Protease Assay



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 our chemically stabilized MSG-Trypsin™ for use as a general protease control; however, other specific protease standard controls can be used. MSG-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.

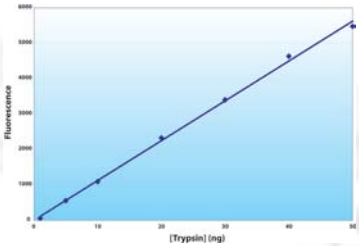


Figure 2: The graph depicts the linear response of the Fluoro™ Protease Assay with increasing concentrations of trypsin protease.

Protease Assay Substrates

RESORUFIN-CASEIN PROTEASE SUBSTRATE

Cat. # 786-321

A colorimetric substrate that when treated with proteases releases resorufin that has an absorbance of 570nm. Supplied lyophilized.

FITC-CASEIN PROTEASE SUBSTRATE

Cat. # 786-322

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.

Phosphatase Inhibitor Cocktails

A large number of biological pathways are controlled by phosphorylation and dephosphorylation of proteins. The cellular processes that are mediated include signal transduction, cell division, cell proliferation and apoptosis. Phosphorylation of a protein occurs when a serine, a threonine, or a tyrosine residue is modified by a protein kinase enzyme by the addition of a phosphate group. The attachment of a phosphate group changes the conformation of the protein and affects its activity. To restore the protein to its original dephosphorylated state, the phosphate group is removed by protein phosphatases.

It is important that the phosphorylation state of the proteins is preserved during extraction of phosphorylated proteins from tissue and cell samples. G-Biosciences offers four different phosphatase inhibitor cocktails, PhosphataseArrest™ I-IV, that help protect protein phosphatase groups.

PhosphataseArrest™ Phosphatase Inhibitor Cocktails

Selection of cocktails to protect protein phosphatase groups

The PhosphataseArrest™ phosphatase inhibitor cocktails are ready-to-use 100X solutions that are simply added to your extraction buffers or samples.

FEATURES

- Single 100X solution
- Ready-to-use.
- Compatible with most phosphatase assays.
- No resuspension required.

PhosphataseArrest™ *I* Cat. # 786-450

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 Cat. # 786-451

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 phosphatase, Alkaline phosphatase
Sodium Tartrate	230.08	Acid phosphatases
Imidazole	68.08	Alkaline phosphatases
Sodium Molybdate	205.92	Acid Phosphatase

PhosphataseArrest™ III Cat. # 786-452

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

PhosphataseArrest™ III is a stable, convenient 100X solution of cantharidin, *p*-bromotetramisole, and microcystin LR.

Phosphatase Inhibitor	M.W.	Target Phosphatases
Cantharidin	196.2	Serine/Threonine phosphatase
p-Bromotetramisole Oxalate	373.23	Alkaline phosphatase
Microcystin LR	995.2	Alkaline phosphatase

PhosphataseArrest™ IV Cat. # 786-602

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

PhosphataseArrest™ IV is a stable, convenient solution of cantharidin, *p*-bromotetramisole oxalate and calyculin.

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

Selection Guide for Phosphatase Inhibitor Cocktails

Cat. #	Phosphatase Inhibitor Cocktail	Target
786-450	PhosphataseArrest™ I	Serine/Threonine, Tyrosine & dual specificity phosphatases
786-451	PhosphataseArrest™ II	Acid, Alkaline & Tyrosine phosphatases
786-452	PhosphataseArrest™ III	Alkaline & Serine/Threonine Phosphatases
786-602	PhosphataseArrest™ IV	Alkaline & Serine/Threonine Phosphatases

Protein Extraction & Lysis Buffer 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.

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), and strong chaotropic extraction buffers that are 2D compatible (FOCUS™ Extraction Buffers).

Common lysis buffers (RIPA), extraction tools (grinding resins), enzyme preparations in a ready-to-use format (lysozyme and Zymolyase®) and other extraction accessories are also offered to assist protein extraction and isolation procedures.

PE LB™ Systems

For isolation of biologically active, soluble proteins.

Bacterial PE LB™ Cat. # 786-176, 786-177

For extraction of biologically active, soluble proteins and inclusion bodies from bacteria. Offered as a kit with lysozyme or as buffer only.

Yeast PE LB™ Cat. # 786-178, 786-179

For extraction of biologically active, yeast soluble proteins, also available with Zymolyase®.

Insect PE LB™ Cat. # 786-411

For extraction of biologically active, proteins from cultured insect cells.

Mammalian Cell PE LB™ Cat. # 786-180

For extraction of biologically active, proteins from tissue cultured mammalian cells.

Tissue PE LB™ Cat. # 786-181

For extraction of biologically active, proteins from fresh or frozen animal tissues.

Lysis Kits & Buffers

Total Protein Extraction™ (TPE™)

Cat. # 786-225

Universal lysis system for the solubilization of total proteins from animal, plant, yeast, bacteria, and other biological samples for SDS-PAGE Analysis.

RIPA Lysis & Extraction Buffer

Cat. # 786-489, 786-490

A complete lysis buffer for the release of cytoplasmic, membrane and nuclear proteins from adherent and suspension cultured mammalian cells. It is fully compatible with our range of individual protease inhibitors and cocktails.

IBS™ Buffer Cat. # 786-183

Specifically developed for solubilization of inclusion buffers.

IBS-HP™ Buffer Cat. # 786-183HP

For the solubilization of inclusion bodies containing highly hydrophobic proteins.

Protein Extraction & Isolation Accessories

EZ-Grind™ Cat. # 786-139

A highly efficient grinding resin that is pre-aliquoted into 1.5ml grinding tubes and is supplied with mathching pestles. The resin is designed for optimal grinding of biological samples for the extraction of both proteins and DNA.

Molecular Grinding Resin™ Cat. # 786-138

Available with Matching Pestles & Tubes

Ideal for grinding small samples and the subsequent preparation of proteins and nucleic acids.

LongLife™ Enzyme Preparations

Ready-to-use enzyme preparations with a long shelf life. The following enzymes are available:

LongLife™ Zymolyase® Cat. # 786-036

For the digestion of yeast and fungal cell walls.

LongLife™ Lysozyme Cat. # 786-037

For the digestion of bacterial cell walls.

LongLife™ PE LB Lysozyme Cat. # 786-042

For the digestion of bacterial cell walls and fully compatible with the PE LB buffer system. Resuces viscosity build-up due to presence of nucleases.

LongLife™ Proteinase K Cat. # 786-038

For the digestion of proteins in nucleic acid preparations.

LongLife™ Nuclease Cat. # 786-039

For the removal of nucleic acids.

LongLife™ RNase Cat. # 786-040

For the digestion of RNA.

LongLife™ DNase Cat. # 786-041

For the digestion of DNA.

PROTEASES

A selection of specific proteases are available that are designed for use in peptide fragmentation for mass spectrometry analysis or protein sequencing. They include mass spectrometry grade modified and stabilized trypsin as well as a selection of sequencing grade endopeptidases for protein sequencing.

MSG-Trypsin™ Cat. # 786-245, 786-245B

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 arginyl-proline and lysyl-proline bonds.

Trypsin undergoes autolysis, producing trypsin fragments that interfere with sequence analysis. G-Biosciences' MSG-Trypsin™ is a chemically modified trypsin that is enzymatically active and yet resistant to autolysis. MSG-Trypsin™ is methylated, TPCK treated and quality tested for mass spectrometry (Figure 3).

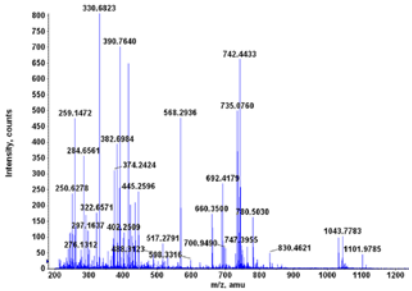


Figure 3: MALDI-TOF Mass Spectrum of casein digested with MASG-Trypsin™ (porcine).

Unlike other trypsin preparations, MSG-Trypsin™ is highly stable (Figure 4), maintaining its activity in severe denaturing buffers (Figure 5) and as a result, is shipped without requiring dry ice and can be stored for a long period without any loss of activity (Figure 4).

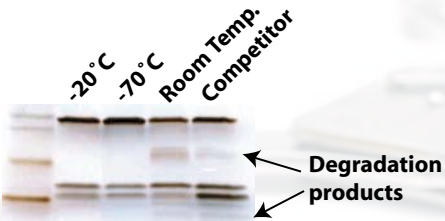


Figure 4: MSG-Trypsin™ is highly stable. MSG-Trypsin™ was stored at -20°C, -70°C and room temperature for six months and then resuspended and analyzed by SDS-PAGE and stained with FOCUS™ FAST*silver*™ (Cat. # 786-240). For a comparison, a competitor's trypsin was resuspended according to the manufacturer's protocol and an equivalent amount was analyzed. Only the MSG-Trypsin™ stored at room temperature and the competitor's trypsin showed degradation products.

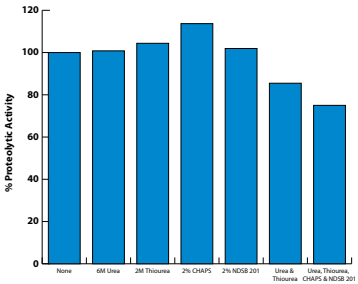


Figure 5: Proteolytic activity of MSG-Trypsin™ in the presence of various denaturants as assessed by our *Protease Screening Kit* (Cat. # 786-137).

We supply two sources of MSG-Trypsin™, either bovine or porcine.

For mass spectrometry sequence analysis, MSG-Trypsin™ to protein ratio of 1:20 to 1:100 is recommended. For convenience, MSG-Trypsin™ is supplied in a 20µg/vial and with a specific resuspension buffer.

Sequencing Grade Proteases

SG-Chymotrypsin™ Cat. # 786-13

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

SG-Chymotrypsin™ is 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.

For protein digestion *SG*-Chymotrypsin™ is added to the protein at a ratio of 1:200 to 1:50, by weight, in a standard digestion buffer. Incubate at 25-30°C for 1 to 10 hours, but can be extended to 24 hours, due to the extended life of the *SG*-Chymotrypsin™. We recommend choosing a ratio of enzyme to protein that allows for the shortest incubation time possible. This will reduce or eliminate the catalyzed hydrolysis of peptide bonds with non-aromatic amino acid residues.

SG-Aspartic-N™ Cat. # 786-12

Hydrolysis of peptide bonds on the N-terminus of aspartate & cysteine residues

SG-Aspartic-N™ is a metallo-endoproteinase, isolated from a mutant strain of *Pseudomonas fragi* that specifically hydrolyzes peptide bonds on the N-terminal side of aspartic acid and cysteine residues. It has an optimal activity at pH6.5-8.0.

SG-Aspartic-N™ is subjected to extensive purification to remove contaminating proteases, which could affect the specificity of the digestion process. The highly purified *SG*-Aspartic-N™ is subsequently modified chemically, resulting in increased resistance to autolysis and improved stability.

For protein fragmentation the enzyme is added to the protein to be digested at a ratio of 1:100 to 1:50, by weight in a standard digestion buffer (50mM Tris.HCl, pH 8.0, 50mM sodium phosphate pH 8.0, or 50mM $(NH_4)HCO_3$). Ideally incubate at 25-30°C for 2 to 6 hours; but can be extended to 24 hours if required. For overnight incubation a ratio of 1:100, enzyme to protein is adequate for most proteins.

SG-Glutamic-C™ Cat. # 786-15

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

SG-Glutamic-C™ is a serine endopeptidase, from *S. 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.

SG-Arginine-C™ Cat. # 786-11

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

SG-Arginine-C™ endopeptidase (Clostripain, from *C. 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-C™ 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).

SG-Lysine-C™ Cat. # 786-14

Cleaves peptide bonds at the carboxy side of lysine

SG-Lysine-C™ 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™ are chemically modified making the enzyme resistant to autolysis and stabilizing its enzymatic activity.

SG-Lysine-C™ 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.

PROTEIN SEQUENCING & MASS SPECTROMETRY ANALYSIS TOOLS

FOCUS™ FAST*silver*™ Cat. # 786-240

Mass Spectrometry Compatible Silver Stain for Enhanced Spot Visualization

FOCUS™ FAST*silver*™ produces crystal clear backgrounds and maximal peptide recovery needed for critical analysis by mass spectrometry.

For mass spectrometry analysis, complete proteolytic digestion and recovery of peptides is required for optimal analysis, however silver ions in traditional silver staining kits inhibit proteolytic digestion. In addition, glutaraldehyde, a common sensitizer in silver stains, modifies peptide lysine residues preventing complete digestion and recovery.

FOCUS™ FAST*silver*™ produces high quality silver staining without the use of glutaraldehyde and is supplied with a highly efficient silver ion removal reagent, *Silver*OUT™. *Silver*OUT™ removes silver ions, which permits complete peptide digestion and extraction of peptides for maximal recovery.

Figure 6: A 2D electrophoresis gel stained with FOCUS™ FAST*silver*™.

FEATURES

- **Full & Efficient Protease Digestion:** Supplied with *Silver*OUT™ to completely remove silver ions that inhibit protease digestion by binding at the active sites of various proteases. This allows for optimal protease digestion.
- **Full and Efficient Recovery of Peptides:** A glutaraldehyde-free silver stain that results in no lysine modification, protein cross linking or reduced peptide recovery. FOCUS™ FAST*Silver*™ allows enhanced protease digestion and efficient recovery of digested peptides.
- **Sensitivity:** ~0.3ng protein and crystal clear background for maximum sensitivity.
- **Short protocol time:** Protein can be detected in less than 90 minutes.

InGel™ Cat. # 786-241

For in gel digestion of protein spots for mass spectrometry analysis. Fully mass spectrometry compatible.

A reliable method for the proteolytic digestion of proteins in gel for subsequent analysis by mass spectrometry.

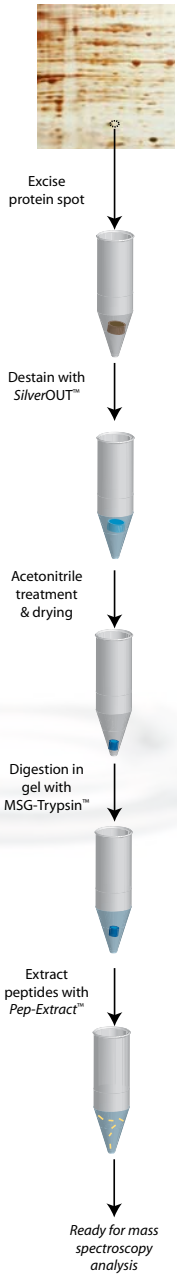
The protein spots are first excised from the gel and transferred to a proteomic grade tube (see accessories). Silver stained gel pieces are washed with *Silver*OUT™ to remove inhibitory silver ions and then the gel pieces are treated with our proprietary Trypsin-Digestion Buffer Mix, a mixture of MSG-Trypsin™ and an optimal digestion buffer, which ensures reliable and efficient protein digestion.

The resulting 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.

The *InGel*™ kit is supplied with all the necessary reagents for 100 protein spots and includes:

- *Silver*OUT™ destaining reagent.
- MSG-Trypsin™, a mass spectrometry grade trypsin with minimal autolysis and increases stability.
- Trypsin Digestion Buffer for optimal trypsin activity.
- *Pep-Extract*™ Buffer, for high level peptide extraction.

These components are also available individually for added convenience.



InGel™ Array Cat. # 786-241A

High throughput in gel digestion of protein spots for mass spectrometry.

A 96-well format kit suitable for processing larger numbers of protein spots concurrently and compatible with spot-picking instruments. Fewer than 96 samples a batch may also be processed without any waste.

Excise protein spots and transfer to the supplied proteomic grade titer plate, destain silver stained gel pieces with *Silver*OUT™ and then incubate with our proprietary Trypsin-Digestion Buffer Mix, a mixture of MSG-Trypsin™ and an optimal digestion buffer, which ensures reliable and efficient protein digestion.

The resulting digested peptides are extracted with *Pep-Extract*™, a high diffusion peptide extraction buffer. The extracted peptides are suitable for mass spectrometry analysis without the need for further cleaning procedures.

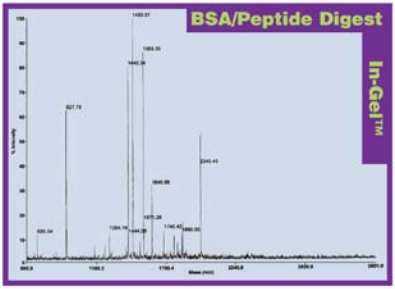


Figure 7: Analysis on BSA with *InGel*™. BSA was resolved on a 2D electrophoresis gel and stained with FOCUS™ FAST*silver*™. The resulting spot was excised and treated with the *InGel*™ *kit*. The resulting peptides were treated to mass spectrometry.

Each kit is supplied with:

- *Silver*OUT™ (Cat. # 786-244) destaining reagent.
- Trypsin Digestion Buffer (Cat. # 786-242) for optimal trypsin activity.
- *Pep-Extract*™ Buffer (Cat. # 786-243) for high level peptide extraction.
- *InGel*™ Array titer plates and caps

The above components are also available individually.

Protein Extraction Kits

One of the most important considerations before analysis of a proteome through running 2D gel electrophoresis is the choice of protien solubilization buffers. The suitable buffer must solubilize proteins effectively, without disturbing the native charge of the proteins. G-Biosciences has developed a range of dry urea based, pre-mixed and ready-to-use solubilization buffers, FOCUS™ Extraction Buffers. These buffers are suitable for sample extraction and solubilization for 2D gel electrophoresis and other applications.

An effective proteome analysis requires the preparation of a sample to bring the wide range of protein species into the dynamic range of detection. G-Biosciences offers a range of FOCUS™ Proteome Kits that are suitable for biological samples from tissues, cells, plants, yeast, bacteria, insects and biological samples. They are supplied with a strong proprietary chaotropic extraction buffer to solubilize proteins. The FOCUS™ Proteome Kits are suitable for the analysis of proteins using electrophoresis and other biochemical techniques.

FOCUS™ Extraction Buffers

Cat. # 786-220, 786-221, 786-222, 786-223, 786-219, 786-233

Strong chaotropic buffers for total protein extraction.

FOCUS™ Extraction Buffers are strong chaotropic buffers that contain optimized concentration of critical agents, buffering and stabilizing agents. The FOCUS™ Extraction Buffers are designed to produce optimal protein extraction and improved spot resolution for 2D gel analysis. Six FOCUS™ Extraction Buffers are available to choose from: FOCUS™ Extraction Buffer- I, II, III, IV, V, or VI.

FOCUS™ Proteome Kits

For the extraction of total proteins.

FOCUS™ Mammalian Proteome

Cat. # 786-246

For extraction of total proteins from cultured mammalian cells and animal tissues.

FOCUS™ Yeast Proteome Cat. # 786-257

Extracts and solubilizes nearly all of the proteins from yeast, including membrane proteins.

FOCUS™ Insect Proteome Cat. # 786-360

Extracts and solubilizes the majority of proteins from insect cells.

FOCUS™ Bacterial Proteome Cat. # 786-258

Developed for the extraction of total soluble proteins and inclusion bodies from bacterial cells.

FOCUS™ Plant Proteome Cat. # 786-259

Designed for plant research and supplied with specific reagents, including reagents to remove pigments and other natural products that may interfer with protein analysis.

BIOSCIENCES®

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