Roche Applied Science

The Complete Guide for Protease Inhibition

c∅mplete protection...
c∅mplete convenience

Proteases are ubiquitous in all living cells. As soon as cells are disrupted, proteases are released and can quickly degrade any protein. This can drastically reduce the yield of protein during isolation and purification. Contaminating proteases can be inhibited by protease inhibitors, thereby protecting the protein of interest from degradation.

The Complete Guide for Protease Inhibition from Roche Applied Science is a comprehensive resource to help you select the appropriate protease inhibitors for your applications. This brochure includes information regarding the specificity, stability, effectiveness, and safety of our protease inhibitors.

Visit ***www.roche-applied-science.com/proteaseinhibitor*** to learn more about protease inhibition, and access technical information about our protease inhibitors, including tips on when and how to use the products.

Protease Inhibitors from Roche Applied Science —

cOmplete protection... cOmplete convenience

Cells contain many different types of proteases. Therefore, mixtures of different inhibitors are needed for complete protection of proteins during isolation and purification for subsequent experiments (*e.g.*, western blotting, reporter gene analysis, or protein interaction or activity assays).

Roche Applied Science has extensive experience with the isolation, purification, and analysis of many different proteins, and with the best ways to protect these proteins from degradation. Choose an individual protease inhibitor for your special application, or achieve broad-spectrum protection with the convenient cOmplete Protease Inhibitor Cocktail Tablets.

Convenient — Choose from a wide range of water-soluble protease inhibitors for exceptional ease of use.

Safe — Use non-toxic inhibitors, such as the cOmplete Protease Inhibitor Cocktail Tablets, Pefabloc SC, and Pefabloc SC PLUS, to achieve protease inhibition without risk to you, or those around you.

Reliable — Count on our expertise in the production of high-quality, function-tested protease inhibitors that have been successfully used by researchers worldwide and referenced in thousands of publications.

Classes of Protease Inhibitors available from Roche Applied Science

	General inhibitors for			
Protease Inhibitor	**Serine proteases**[a]	**Cysteine proteases**[b]	**Metallo-proteases**[c]	**Aspartic proteases**[d]
Aprotinin		E-64	Phosphoramidon	Pepstatin
Pefabloc SC and Pefabloc SC PLUS			Bestatin (aminopeptidases)	
Leupeptin *(inhibits serine and cysteine proteases with trypsin-like specificity)*				
PMSF				
cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets*				
cOmplete Protease Inhibitor Cocktail Tablets*				
α_2-Macroglobulin				

* When extractions or single-step isolations are necessary in the acidic pH range, include Pepstatin along with cOmplete tablets to ensure aspartic (acid) protease inhibition.

a) Contain serine and histidine in the active center

b) Contain cysteine (thiol, SH-) in the active center

c) Contain metal ions (*e.g.*, Zn^{2+}, Ca^{2+}, Mn^{2+}) in the active center

d) Contain aspartic (acidic) group in the active center

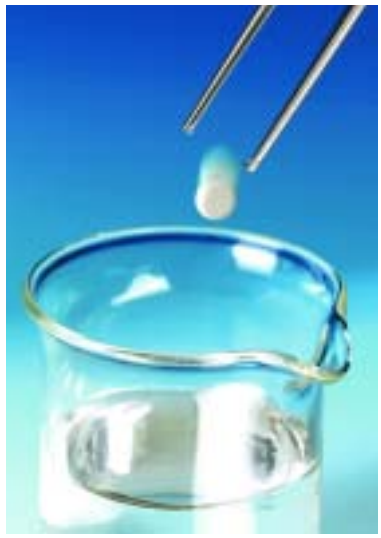
Protease-specific inhibitors	for the inhibition of:
Antipain dihydrochloride	Papain, Trypsin (Plasmin)
Calpain Inhibitor I	Calpain I > Calpain II
Calpain Inhibitor II	Calpain II > Calpain I
Chymostatin	Chymotrypsin
TLCK	Trypsin, other serine and cysteine proteases (*e.g.*, Bromelain, Ficin, Papain)
Trypsin-Inhibitor (chicken egg white, soybean)	Trypsin

Simplify protease inhibition with convenience and reliability in a c𝒪mplete tablet

When isolating or purifying proteins, benefit from the ultimate in convenience – use c𝒪mplete Protease Inhibitor Cocktail Tablets and eliminate the time-consuming search for the right protease inhibitor. c𝒪mplete is a proprietary blend of protease inhibitors, formulated as a ready-to-use water-soluble tablet. Simply add the convenient tablet to your homogenization buffer, and instantly protect your proteins against a broad range of proteases.

Obtain the protection you need, with convenience and reliability — choose our easy-to-use c𝒪mplete Protease Inhibitor Cocktail Tablets.

Convenience



- Consistently inhibit a multitude of protease classes (Table 1), including serine proteases, cysteine proteases, and metalloproteases.
- Inhibit proteolytic activity in extracts from almost any tissue or cell type, including animals, plants, yeast, bacteria, and fungi (for examples, see page 5).
- Choose from two available tablet sizes, with or without EDTA, for 10 or 50 ml of lysate.
- Drop a tablet into your lysis buffer and eliminate the cumbersome job of weighing small amounts of different protease inhibitors on an analytical scale, and dissolving the mix in DMSO.

Reliability

- Deliver consistent doses of protease inhibition.
- Obtain stable, non-toxic protection in aqueous buffers.
- Maintain the stability of metal-dependent proteins, and function of purification techniques (*i.e.*, IMAC [immobilized metal affinity chromatography] for isolation of Poly-His-tagged proteins) by using EDTA-free c𝒪mplete Protease Inhibitor Tablets.

Count on c𝒪mplete protection to eliminate the worry

Achieve broad-spectrum protection with a single tablet. c𝒪mplete Protease Inhibitor Cocktail Tablets eliminate the questions... and the doubt.

▶ **Table 1: Inhibition of different proteases by c𝒪mplete Protease Inhibitor Tablets.** One c𝒪mplete tablet was added per 50 ml incubation solution. Proteolytic activity was determined with the Roche Applied Science Universal Protease Substrate (casein, resorufin-labeled), Cat. No. 11 080 733 001. When extractions or single-step isolations are necessary in the acid pH range, simply include Pepstatin along with c𝒪mplete tablets to ensure aspartic (acid) protease inhibition. All experiments were performed at room temperature.

Source and concentration of protease	Type of protease	% Inhibition immediately after adding c𝒪mplete	% Inhibition 60 minutes after adding c𝒪mplete
Chymotrypsin, 1.5 µg/ml	Serine	97%	97%
Thermolysin, 0.8 µg/ml	Metallo	99%	100%
Papain, 1 mg/ml	Cysteine	95%	73%
Pronase, 1.5 µg/ml	Mixture	88%	99%
Pancreatic extract, 1.5 µg/ml	Mixture	87%	99%
Trypsin, 0.002 µg/ml	Serine	93%	73%

Choose c𝒪mplete inhibition

Select the appropriate c𝒪mplete Protease Inhibitor Cocktail Tablet for protection against unwanted protease activity in your application. Benefit from multiple tablet format options to meet your needs — choose from two tablet sizes (regular or mini), with or without EDTA. The choice is yours!

◀ **Table 2: Choose the correct c𝒪mplete tablet for your application.**

Application	c𝒪mplete tablets	c𝒪mplete, EDTA-free	c𝒪mplete, Mini	c𝒪mplete, EDTA-free
Inhibition during initial extraction steps (volumes > 50 ml)	++	++	+	+
Inhibition during subsequent purification protocols (volumes < 50 ml)	+[1]	+[1]	++	++
Inhibition during subsequent purification steps require free divalent cations for further processing[2]	0	++	0	++
Samples containing high metalloproteolytic activity	++	0	++	0

++ Product of choice
\+ Can also be used
0 Not recommended

1 Preparation of stock solutions is recommended.
2 Important, for example, with metal-chelate chromatography purification of Poly-His-tagged proteins, or protein samples used for signal transduction research.

Achieve c𝒪mplete success

Choose our versatile c𝒪mplete Protease Inhibitor Cocktail Tablets to provide the protease inhibition you need. Try the c𝒪mplete Protease Inhibitor Cocktail Tablets today, and see how simple success can be. Your laboratory just isn't complete without it.

Some examples of cells, tissues, and organisms in which protease activity has been successfully inhibited with c𝒪mplete tablets — as reported in scientific literature:

- *Acintobacillus actinomycetemcomitans*
- Adipocytes (mouse, rat)
- Adrenal gland (PC-12, rat)
- Bladder carcinoma cells (T24, human)
- Bone marrow cells (mouse, human)
- Bone osteosarcoma (U-2 OS, SaOs-2, human)
- Brain neuroblastoma cells (SK-N-BE(2), human)
- Brain tissue (bovine, mouse, rat, human)
- Breast cancer cells (BT20, MCF7, human)
- Bronchial Alveolar Lavage Fluid (mouse, rat)
- Bronchial Biopsies (human)
- Bronchial epithelial cell line (BZR, human)
- Cardiomyocytes (mouse, rat)
- Cervix adenocarcinoma (HeLa, human)
- Cochlea (rat)
- Colon carcinoma cells (T84, human)
- Colorectal adenocarcinoma cells (CaCo-2) (human)
- Colorectal and duodenal adenomas
- Colorectal carcinoma cells (HCT-116, human)
- Cortex (rat)
- Dictyostelium (amoeba)
- *E. coli*
- Endothelial cell line
- Epidermis (human)
- Epithelial cell lines (human, bovine)
- Fat (mouse)
- Fibroblasts (human; NIH-3T3, MDTF, mouse)
- Fibrosarcoma cell line (HT1080, human)
- Fruit (tomato)
- Glioblastoma cell line (U87MG)
- Head (*Drosophila*)
- Heart (human, mouse, chicken)
- Hematopoietic cell lines (mouse, human)
- Immature seed (soy)
- Insect cell lines (Sf2, Sf21, Sf9, Tn5)
- Keratinocytes (human)
- Kidney (dog, human, mouse, rat, monkey, *Xenopus*)
- Leaf (*Arabidopsis*)
- Liver carcinoma cells (HepG2, Hep3B, human)
- Liver tissue (mouse, rat, *Xenopus*)
- Lung carcinoma cells (A549, human)
- Lung homogenates (mouse, *Xenopus*)
- Lung lavage fluid (mouse)
- Luteal tissue (bovine)
- Lymph nodes (mouse)
- Lymphoblastoids (human)
- Lymphocytes (Jurkat, human; WEHI 3b D, mouse; monkey)
- Mammary carcinoma cells (MDA468, human)
- Mammary epithelial cells (HMEC)
- Mammary gland (mouse)
- Mast cell line (human)
- Monocyte cells (THP-1, human)
- Muscle (*Drosophila*, human, mouse, rat, rabbit, *Xenopus*)
- *Neisseria gonorrhoeae*
- Neurons (rat)
- Ovarian cancer (OVCAR-3, human)
- Ovary cells (CHO, hamster)
- Pancreas (mouse)
- Parathyroid tissue (bovine)
- Peripheral blood cells (BA/F3, mouse; CEM, HL-60, human)
- *Pichia pastoris*
- Placental labyrinth (mouse, rat)
- Platelets (human)
- Primary chondrocytes (human)
- Primary lung cancer cells
- Primary mast cells (mouse)
- Primary neuronal cultures (mouse)
- Prostate adenocarcinoma cells (PC-3, human)
- Prostate carcinoma cells (DU-145 and LNCaP, human)
- *Pseudomonas*
- Rectal tissue (rabbit)
- Renal cell carcinomas (human)
- Reticulocyte lysate (rabbit)
- Retina (mammalian)
- *Saccharomyces cerevisiae*
- Salivary gland (mouse)
- *Salmonella typhimurium*
- Seed (*Arabidopsis*)
- Skin (human)
- Spermatogenic cells (mouse)
- Spinal cord (rat)
- Spleen (mouse, rat, *Xenopus*)
- *Staphylococcus aureus*
- *Streptococcus pneumoniae*
- Superior cervical ganglion (mouse)
- *Toxoplasma gondii*
- Umbilical vein endothelial cells (HUVEC, human)
- Whole plant tissue

Protease Inhibitor Cocktails

Inhibitor	Specificity of inhibitor	Solubility / Stability
cOmplete Protease Inhibitor Cocktail Tablets (1 tablet used in 50 ml) 11 697 498 001 20 tablets 11 836 145 001 3 x 20 tablets	A proprietary mixture of several protease inhibitors with broad inhibitory specificity. Inhibits serine, cysteine, and metalloproteases, as well as calpains. Use for extracts from tissues or cells, including animals, plants, bacteria, yeast, and fungi.	Soluble in aqueous buffers, or add directly to extraction media. Alternatively, prepare 25x stock solutions in 2 ml water or 100 mM phosphate buffer, pH 7.0. Stock solution is stable for 1-2 weeks at 2-8°C or at least 12 weeks at -15 to -25°C. Can be used in thiol-containing solutions at room temperature.
cOmplete, Mini Protease Inhibitor Cocktail Tablets (1 tablet used in 10 ml) 11 836 153 001 25 tablets	See specificity for cOmplete tablets above.	Soluble in aqueous buffers, or add directly to extraction media. Alternatively, prepare 7x stock solutions in 1.5 ml water or 100 mM phosphate buffer, pH 7.0. Stock solution is stable for 1-2 weeks at 2-8°C or at least 12 weeks at -15 to -25°C. Can be used in thiol-containing solutions at room temperature.
cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets (1 tablet used in 50 ml) 11 873 580 001 20 tablets	A proprietary mixture of several protease inhibitors that inhibit a broad spectrum of serine and cysteine proteases. Use for extracts from tissue or cells including animals, plants, bacteria, yeast, and fungi. EDTA-free tablets will not affect the stability or function of metal-dependent proteins.	Soluble in aqueous buffers, or add directly to extraction media. Alternatively, prepare 25x stock solutions in 2 ml water or 100 mM phosphate buffer, pH 7.0. Stock solution is stable for 1-2 weeks at 2-8°C or at least 12 weeks at -15 to -25°C. Can be used in thiol-containing solutions at room temperature.
cOmplete, Mini, EDTA-free Protease Inhibitor Cocktail Tablets (1 tablet used in 10 ml) 11 836 170 001 25 tablets	See specificity for cOmplete, EDTA-free tablets above.	Soluble in aqueous buffers, or add directly to extraction media. Alternatively, prepare 7x stock solutions in 1.5 ml water or 100 mM phosphate buffer, pH 7.0. Stock solution is stable for 1-2 weeks at 2-8°C, or at least 12 weeks at -15 to -25°C. Can be used in thiol-containing solutions at room temperature.

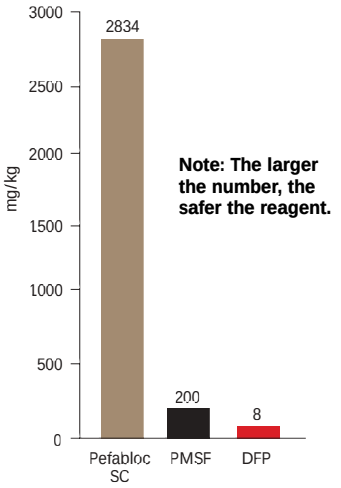
** Aspartic (acid) proteases exhibit pronounced activity only at low pH. If extraction or single isolation steps must be performed at low pH, add Pepstatin to ensure aspartic protease inhibition.

*** If IMAC (immobilized metal chelate affinity chromatography) is to be performed (*e.g.*, for isolating Poly-His-tagged recombinant proteins), remove EDTA via dialysis. As an alternative, use the cOmplete EDTA-free tablets, available separately.

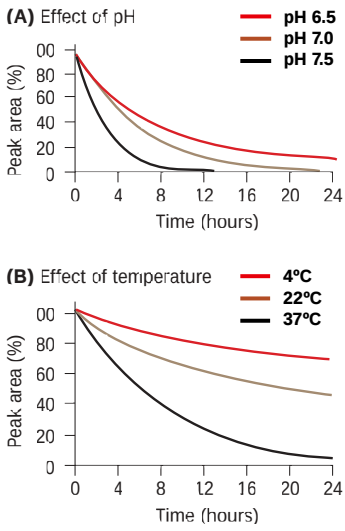
Suggested starting concentration**	Mode of action	Notes
Dissolve one tablet in 50 ml aqueous buffer (without divalent cations) or water. If very high proteolytic activity is present, use one tablet for 25 ml buffer.	Contains both reversible and irreversible protease inhibitors.	■ For optimal inhibition of metalloproteases, do not prepare protease inhibitor cocktails with buffers containing divalent cations (*e.g.*, Ca^{2+}, Mg^{2+}, or Mn^{2+}).**, *** ■ A solution of one c*O*mplete tablet in 50 ml water has an absorbance of 0.08 at 280 nm. ■ All inhibitors in c*O*mplete tablets can be removed via dialysis. Use of a membrane with cutoff >10 kD is recommended. ■ Does not contain reducing agents such as DTT.
Dissolve one tablet in 10 ml aqueous buffer or water. If very high proteolytic activity is present, use one tablet for 7 ml buffer.	Contains both reversible and irreversible protease inhibitors.	■ See notes for c*O*mplete tablets above.
Dissolve one tablet in 50 ml aqueous buffer or water. If very high proteolytic activity is present, use one tablet for 25 ml buffer.	Contains both reversible and irreversible protease inhibitors.	■ Does not contain EDTA; thus metal-dependent proteins and IMAC isolation techniques (*e.g.*, for Poly-His-tagged proteins) are not affected.** ■ All inhibitors in c*O*mplete tablets can be removed via dialysis. Use of a membrane with cutoff >10 kD is recommended. ■ Does not contain reducing agents such as DTT.
Dissolve one tablet in 10 ml aqueous buffer or water. If very high proteolytic activity is present, use one tablet for 7 ml buffer.	Contains both reversible and irreversible protease inhibitors.	■ See notes for c*O*mplete, EDTA-free tablets above.

In just minutes, serine proteases can destroy the proteins you have spent days isolating. In the past, PMSF (phenyl-methyl-sulfonyl fluoride) and DFP (diisopropyl fluorophosphate) were used to eliminate this problem. However, due to their poor stability and solubility in aqueous solutions, these inhibitors provided uncertain protection for protein samples. Now, protecting your proteins has a simple solution... an aqueous solution, made with Pefabloc SC.

Obtain convenient and reliable serine protease inhibition with Pefabloc SC



▲ Figure 1. Safety of Pefabloc SC compared to PMSF and DFP.
Mice were given oral doses of each inhibitor, and the LD_{50} in mg/kg was determined.



▲ Figure 2. The effect of temperature and pH on the stability of Pefabloc SC.

Graph (A) shows the relative stability of Pefabloc SC (5.0 mg/ml) in an aqueous phosphate buffer at 37°C.

Graph (B) shows the relative stability of Pefabloc SC (5.0 mg/ml) in an aqueous phosphate buffer at pH 7.0.

Despite the popularity of PMSF and DFP, both have serious disadvantages. Benefit from a safe, effective alternative: choose Pefabloc SC, the preferred serine protease inhibitor, to provide superior protection with unmatched convenience and reliability.

Convenience

- Benefit from an easy-to-use inhibitor — Pefabloc SC is readily soluble in water, and may be added directly to aqueous buffers. Unlike Pefabloc SC, PMSF and DFP are poorly soluble in water. Because of this, stock solutions must be prepared in organic solvents, and only then can be added to aqueous solutions.
- Avoid hazardous compounds — PMSF is a neurotoxin, and DFP is a deadly cholinesterase inhibitor. In contrast, non-toxic Pefabloc SC provides complete protease inhibition without risk to you, or those around you (Figure 1).

Reliability

- Ensure protection with improved stability — Pefabloc SC remains highly active in aqueous solutions, protecting your proteins long after PMSF and DFP have failed. Protease inhibition is sustained even at pH levels above 7.0 and temperatures above 4°C (Figure 2).
- Maximize inhibition — Superior solubility and stability in aqueous buffers mean that Pefabloc SC eliminates the guesswork and promotes success. The poor solubility and stability of PMSF make it difficult to maintain an effective concentration, and leaves you questioning whether levels of active inhibitor are high enough to assure total protection.

Increase flexibility with a broad range of applications

Choose Pefabloc SC for all applications where the general inhibition of serine proteases is desired. With its high stability and irreversible inhibition mechanism, protein solutions are protected throughout procedures, such as:

- extraction processes (from animal tissues or cells, plants, bacteria, yeast, and fungi)
- subsequent purification steps
- varied sample storage conditions
- downstream protein detection (*e.g.*, western blotting, reporter gene analysis, *etc.*)
- biochemical studies where proteins are required.

Use Pefabloc SC to inactivate proteinase K, for example, during pulsed-field gel electrophoresis (PFGE).With this technique, isolating the genomic DNA requires proteinase K to degrade cellular components, and this highly resilient protease is difficult to inactivate. Pefabloc SC inhibits proteinase K, and protects the stability of restriction enzymes used for further DNA analysis.

Benefit from additional convenience and reliability with Pefabloc SC PLUS

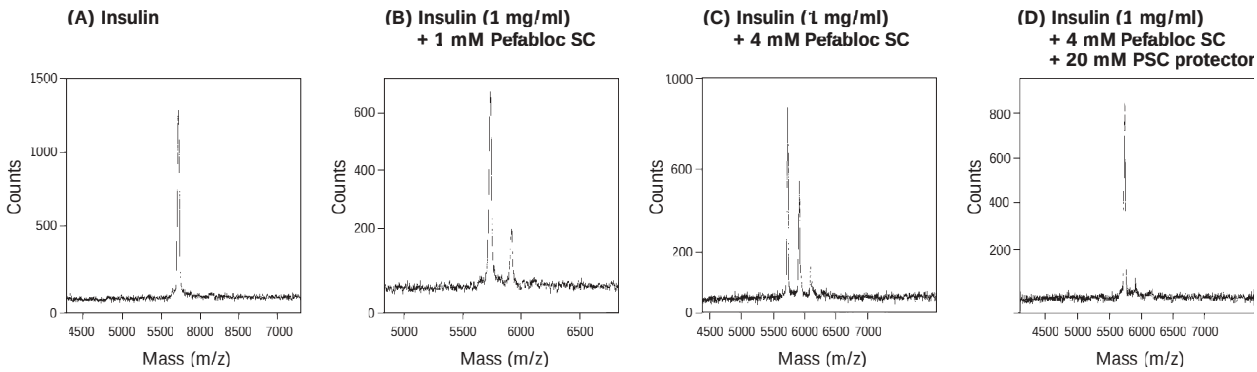
Recent findings indicate that sulfonyl-type serine protease inhibitors such as Pefabloc SC and PMSF can bind covalently to proteins. This can occur when the inhibitors are used in high concentrations, or during extended incubation times under alkaline conditions (Figure 3). This interaction adversely affects the tyrosine and lysine residues of a protein, as well as the free amino terminus. The Pefabloc SC PLUS set combines the protease inhibitor Pefabloc SC with a uniquely formulated Pefabloc SC protector (PSC protector). In addition to the benefits already described for Pefabloc SC, it offers additional convenience and reliability.

Additional Convenience

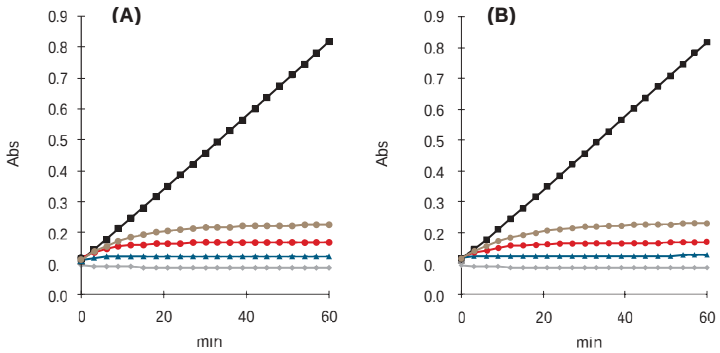
- Take advantage of a simplified, two-reagent system with balanced quantities of reagents.
- Ensure safety — both Pefabloc SC and the Pefabloc SC protector are stable and non-toxic.

Additional Reliability

- Prevent covalent binding between proteins and Pefabloc SC, even at high concentrations, extended incubation times, and at alkaline pH (Figure 3).
- Obtain optimum protection — no influence on the inhibitory effectiveness of Pefabloc SC (Figure 4).



▲ Figure 3 (A-D): Mass spectrograms showing the covalent interaction between insulin and the protease inhibitor Pefabloc SC. Diagram A is the insulin blank. At 1 mM Pefabloc SC, the formation of the binding is visible as a second peak formation (Diagram B). Higher concentrations of the protease inhibitor result in more than one interaction per insulin molecule (Diagram C). The special PSC protector eliminates this covalent interaction, even at the highest concentrations (Diagram D). Matrix peaks are subtracted.



◀ Figure 4. Chymotrypsin inhibition with Pefabloc SC in the absence (A) and presence (B) of the Pefabloc SC protector. Results show no change in the effectiveness of inhibition.

Individual Protease Inhibitors

Inhibitor	Specificity of inhibitor	Solubility/Stability*
Antipain dihydrochloride (Papain Inhibitor) 11 004 646 001 10 mg	Inhibits papain and trypsin. Plasmin is inhibited to a small extent.	Soluble in water, methanol, or DMSO*** to 20 mg/ml. Sparingly soluble in ethanol, propanol, or butanol. Insoluble in benzene, chloroform ($CHCl_3$), hexane, or petroleum and ethyl ethers. Dilute solutions should be stored frozen in aliquots at -15 to -25°C. Stable for approximately 1 month.
Aprotinin 10 236 624 001 10 mg 10 981 532 001 50 mg 11 583 794 001 100 mg	Serine protease inhibitor. Does not act on thrombin or Factor X. Inhibits plasmin, kallikrein, trypsin, and chymotrypsin with high activity.	Freely soluble in water (10 mg/ml) or aqueous buffer solution (*e.g.*, Tris, 0.1 M, pH 8.0). A solution adjusted to pH 7–8 is stable for approximately 1 week at 2-8°C. Aliquots stored at -15 to -25°C are stable for approximately 6 months.
Bestatin [(2S, 3R)-3-Amino-2-hydroxy-4-phenylbutanoyl]-L-leucine hydrochloride 10 874 515 001 10 mg	Primarily, if not exclusively, an inhibitor of amino peptidases and other exopeptidases, including aminopeptidases found in wheat germ and reticulocyte lysate *in vitro* translation systems (*e.g.*, aminopeptidase B, leucine aminopeptidase, tripeptide amino-peptidase, and aminopeptidases on the surface of mammalian cells). It does not inhibit carboxypeptidases.	Soluble to 20 mg/ml in 1 M HCl, 5 mg/ml in methanol, and 1 mg/ml in 0.15 M NaCl. Do not store in HCl. We recommend a stock solution of 2–5 mg/ml in methanol. Solutions are stable for 6 months if stored in aliquots at -15 to -25°C.
Calpain Inhibitor I (N-Acetyl-Leu-Leu-norleucinal), synthetic 11 086 090 001 25 mg	Inhibitor of calpains. Calpains are calcium-dependent neutral cysteine proteases. Inhibits activity of Calpain I. LD_{50} for 0.02 U platelet Calpain I: 0.05 µmol/l. Some inhibitory activity against Calpain II. Inhibits papain to a lesser extent.	Soluble in DMF, ethanol, or methanol to 10 mg/ml. For a stock solution, we recommend dissolving 1 mg of the inhibitor in 100 µl DMF, methanol or ethanol. Before use, dilute with water or phosphate buffer (0.1 M, pH 7.5) to desired concentration. Solutions in DMF, ethanol, or methanol are stable for 2–3 days at 2-8°C and approximately 4 weeks at -15 to -25°C. We recommend making solutions up fresh before use.
Calpain Inhibitor II (N-Acetyl-Leu-Leu-methioninal), synthetic 11 086 103 001 25 mg	Inhibits activity of Calpain II. Inhibits Calpain I (LD_{50} = 0.12 µmol/l) and papain to a lesser extent.	(See Calpain Inhibitor I, above).
Chymostatin 11 004 638 001 10 mg	Specific inhibitor of α-, β-, γ-, δ-chymotrypsin.	Soluble in glacial acetic acid, or DMSO*** to 20 mg/ml. Sparingly soluble in water, methanol, or ethanol. Insoluble in ethyl acetate, petroleum and ethyl ethers, hexane, or chloroform ($CHCl_3$). Dilute solutions should be stored frozen in aliquots at -15 to -25°C. Stable for approximately 1 month.
E-64 (N-(N-(L-3-Trans-carboxirane-2-carbonyl)-L-leucyl)-agmatine) 10 874 523 001 10 mg 11 585 681 001 25 mg	Inhibits papain and other cysteine proteases like cathepsin B and L.	Soluble to 20 mg/ml in a 1:1 (v/v) mixture of ethanol and water. Solutions are stable for 1 month if stored in aliquots at -15 to -25°C.
Leupeptin Ac-Leu-Leu-arginal x 1/2 H_2SO_4, synthetic 11 017 101 001 5 mg 11 017 128 001 25 mg 11 034 626 001 50 mg 11 529 048 001 100 mg	Inhibits serine and cysteine proteases such as trypsin, papain, plasmin, and cathepsin B.	Highly soluble in water (1 mg/ml). Stable for at least 1 week at 2-8°C and 6 months frozen in aliquots at -15 to -25°C.

* Unless otherwise stated, make solutions of inhibitors fresh daily.

** Recommended as a starting concentration. Suitable concentrations must be determined empirically for each new system.

*** CAUTION: DMSO (Dimethyl sulfoxide) will permeate the skin, carrying solubilized protease inhibitors. Always wear appropriate protection for eyes, skin, *etc*.

Suggested starting concentration**	Mode of action of inhibitor	Notes
50 µg/ml (74 µM) (1 U of papain is inhibited to 49% by 0.9 µg of antipain.)	Reversible	■ Molecular Weight: 677.63 ■ Antipain is more specific for papain and trypsin than is leupeptin. ■ The inhibitory potency of antipain is 100-fold higher than that of elastatinal.
0.06–2.0 µg/ml (0.01–0.3 µM)	Reversible	■ Molecular Weight: 6,512 ■ Avoid repeated freeze-thaws and exposure to strong alkali solutions. ■ Aprotinin is inactive at pH >12.8.
40 µg/ml (130 µM)	Reversible	■ Molecular Weight: 308.4 ■ Bestatin has been found to have antitumor properties and enhances not only blastogenesis and lymphocytes *in vitro*, but also establishes a delayed-type hypersensitivity *in vivo*.
17 µg/ml	Reversible	■ Molecular Weight: 383.5 ■ Not soluble in water.
7 µg/ml	Reversible	■ Molecular Weight: 401.6 ■ Not soluble in water.
6–60 µg/ml (10–100 µM) Unit definition: One unit chymo-trypsin is inhibited to 49% by 1.8 µg of chymostatin.	Reversible	■ Molecular Weight: 607.71
0.5–10 µg/ml (1.4–28.0 µM)	Irreversible	■ Molecular Weight: 357.4 ■ Stable between pH 2–10. ■ Unstable in strong alkali and strong mineral acids.
0.5 µg/ml (1 µM)	Irreversible	■ Molecular Weight: $C_{20}H_{38}N_6O_4 \times {}^1/_2\ H_2SO_4$: 475.6 $C_{20}H_{38}N_6O_4 \times {}^1/_2\ H_2SO_4 \times H_2O$: 493.6

Individual Protease Inhibitors

Inhibitor	Specificity of inhibitor	Solubility/Stability*
α_2-Macroglobulin 10 602 442 001 25 Inh. U	A general endoproteinase inhibitor. Inhibits most endoproteinases, but does not inhibit endoproteinases that are highly specific for one or a limited number of sequences (*e.g.*, tissue kallikrein, urokinase, coagulation factor XIIa, and endoproteinase Lys-C).	Soluble in water. Stable for at least 1 week at room temperature or 3 weeks at 2-8°C. Can also be frozen in aliquots at -15 to -25°C, where it remains stable for at least 6 months. Sensitive to acidic pH, denatured below pH 4.0. Ammonia methylamine and hydroxylamine (above pH 7.0) cause irreversible conversion to the inactive form.
Pefabloc SC **4-(2-Aminoethyl)-benzenesulfonyl-fluoride, hydrochloride (AEBSF)** 11 429 868 001 100 mg 11 585 916 001 500 mg 11 429 876 001 1 g	Irreversibly inhibits serine proteases, including trypsin, chymotrypsin, plasmin, plasma kallikrein, and thrombin.	Soluble up to 100 mg/ml in aqueous buffers or water. Stable in solution for 1–2 months if stored in aliquots at -15 to -25°C. Only slight hydrolysis occurs under weakly basic conditions (pH 8.0–9.0).
Pefabloc SC PLUS 11 873 601 001 set I (100 mg Pefabloc SC) 11 873 628 001 set II (1 g Pefabloc SC)	Specificity of the protease inhibitor remains unchanged. See Pefabloc SC.	Solubility and stability of the protease inhibitor remains unchanged. See Pefabloc SC.
Pepstatin 10 253 286 001 2 mg 11 359 053 001 10 mg 11 524 488 001 50 mg	Inhibits aspartic (acid) proteases such as pepsin, renin, cathepsin D, chymosin, and many microbial acid proteases.	Soluble in methanol to approximately 1 mg/ml. Also soluble to 1 mg/ml in ethanol if allowed to sit overnight, and to 300 µg/ml in 6 N acetic acid. Stable for at least 1 week at 2-8°C, or 1 month if stored in aliquots at -15 to -25°C.
Phosphoramidon **N-(α-Rhamnopyranosyloxyhydroxyphosphinyl)-L-leucyl-L-tryptophan, disodium salt** 10 874 531 001 5 mg	Specifically inhibits thermolysin, collagenase, and metalloendoproteinases from various microorganisms (*Bacillus subtilis, Streptomyces griseus*, and *Pseudomonas aeruginosa)*.	Salts of phosphoramidon are soluble to 20 mg/ml in water. Also soluble in methanol or DMSO.*** Recommended stock solution 1–20 mg/ml. Stable in solution for 1 month if stored in aliquots at -15 to -25°C.
PMSF **(Phenylmethylsulfonyl fluoride)** 10 236 608 001 1 g 10 837 091 001 10 g 11 359 061 001 25 g	Inhibits serine proteases (chymotrypsin, trypsin, and thrombin). Also inhibits cysteine proteases such as papain (reversible by DTT treatment).	Soluble to >10 mg/ml in isopropanol, ethanol, methanol, or 1,2-propanediol. Unstable in aqueous solution. In 100% isopropanol, stable for at least 9 months at +25°C.
TLCK **L-1-Chloro-3-(4-tosyl-amido)-7-amino-2-heptanone hydrochloride, N-α-Tosyl-L-lysine chloromethyl ketone** 10 874 485 001 100 mg	Irreversibly and specifically inhibits trypsin. Also inhibits many other serine and cysteine proteases such as bromelain, ficin, and papain.	Salts of TLCK are soluble to 20 mg/ml in water. We recommend a stock solution of 1 mg/ml in either dilute (1 mM) HCl or buffer, pH < 6; to ensure stability (see "Notes" column).
Trypsin Inhibitors from chicken egg white 10 109 878 001 1 g **from soybean** 10 109 886 001 50 mg	Inhibits trypsin. Soybean trypsin inhibitor also inhibits factor Xa, plasmin, and plasma kallikrein. Neither inhibits metallo, cysteine, and aspartic proteases or tissue kallikrein.	Both are soluble in water. Recommended stock solution: 1 mg/ml. Store frozen in aliquots at -15 to -25°C. Stable for at least 6 months.

* Unless otherwise stated, make solutions of inhibitors fresh daily.

** Recommended as a starting concentration. Suitable concentrations must be determined empirically for each new system.

*** CAUTION: DMSO (Dimethyl sulfoxide) will permeate the skin, carrying solubilized protease inhibitors. Always wear appropriate protection for eyes, skin, *etc*.

Suggested starting concentration**	Mode of action of inhibitor	Notes
Unit definition: One inhibitor unit inhibits 9.1 µg of trypsin.	Reversible	■ Molecular Weight: 725,000 ■ Do not use α_2-Macroglobulin in the presence of DTT. DTT, even at 1 mM, causes reversible dissociation into inactive subunits. α_2-Macroglobulin acts by physically entrapping the endoproteinases, usually in a 1:1 ratio.
0.1–1.0 mg/ml (0.4–4 mM)	Irreversible	■ Molecular Weight: 239.5 ■ A safe, stable, and water-soluble alternative to PMSF and DFP.
0.1–1.0 mg/ml (0.4–4.0 mM)	Irreversible	■ Sets contain of Pefabloc SC and a special protector (PSC-protector). ■ The set eliminates interaction between Pefabloc SC and sample proteins.
0.7 µg/ml (1 µM)	Reversible	■ Molecular Weight: 685.9 ■ Insoluble in water.
4–330 µg/ml (7–570 µM)	Irreversible	■ Molecular Weight: 579.6
17–170 µg/ml (0.1–1 mM)	Irreversible	■ Molecular Weight: 174.2 ■ Add fresh PMSF at every isolation/purification step (from stock solution). ■ Does not inhibit metalloproteases, most thiol proteases, and aspartic proteases.
37–50 µg/ml (100–135 µM)	Irreversible	■ Molecular Weight: 369.3 ■ Stable at +25°C pH < 6.0. ■ Rapidly decomposes at pH >7.5. For example, at pH 9.0, +25°C, TLCK's half-life is only 5 minutes. ■ Chymotrypsin is not inhibited.
10–100 µg/ml	Reversible	■ Molecular Weight: (egg white) 28,000 (soybean) 20,100 ■ Egg white inhibitor is stable at acid pH and labile at alkaline pH. ■ Soybean inhibitor is sensitive to heat, high pH, and protein-precipitating solutions.

Protease Inhibitors Set

In certain cases, irregular types of protease activity are encountered. Determining which protease inhibitor to use can be difficult and expensive — unless you use our **Protease Inhibitor Set**. Take advantage of this unique set, consisting of ten different inhibitors, and perform easy, economical screening for the correct inhibitor for your application.

Protease Inhibitors Set Cat. No. 11 206 893 001

Inhibitors included in the set	Specificity of inhibition	Quantity Supplied
Antipain-dihydrochloride	Papain, Trypsin, Cathepsin A and B	3 mg
Aprotinin	Trypsin, Plasmin, Chymotrypsin, Kallikrein	0.5 mg
Bestatin	Aminopeptidases	0.5 mg
Chymostatin	α-, β-, γ-, δ-Chymotrypsin	1 mg
E-64	Cysteine Proteases	3 mg
EDTA-Na_2	Metalloproteases	10 mg
Leupeptin	Serine and Cysteine Proteases such as Plasmin, Trypsin, Papain, Cathepsin B	0.5 mg
Pefabloc SC	Serine Proteases	20 mg
Pepstatin	Aspartic Proteases	0.5 mg
Phosphoramidon	Metalloproteinases, specifically Thermolysin	3 mg

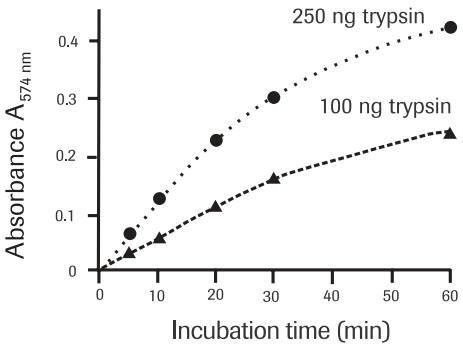
Verify protease inhibition

Use Roche Applied Science's **Universal Protease Substrate** for the rapid and highly sensitive detection of trace protease activity, or to determine the effectiveness of protease inhibition. Protease activity releases resorufin-labeled peptides from the patented substrate, resorufin-labeled casein. The labeled peptides can be measured spectrophotometrically or fluorimetrically in a homogeneous assay. The concentration of these resorufin-labeled peptides in the supernatant is directly related to the proteolytic activity present.

- Conveniently detect nanogram quantities of proteolytic activity in less than one hour.
- Perform highly sensitive protease detection in a homogeneous assay.

Universal Protease Substrate (casein, resorufin-labeled)* **Cat. No.** 11 080 733 001 **Pack Size** 15 mg
11 734 334 001 40 mg

* Patent US 4,954,630 owned by Roche Diagnostics GmbH.



▲ **Figure 5: Influence of the incubation time on the casein-resorufin hydrolysis by trypsin.**

Enzyme	Enzyme amount	$\Delta OD_{574\ nm}$	Enzyme amount	Absorbance ΔE_{574nm}
Pronase†	0.1 µg	0.11	1 mg	1.9
Trypsin, sequencing grade†	0.1 µg	0.07	20 µg	1.06
Endoproteinase Asp-N, sequencing grade†	0.1 µg	0.09	10 µg	1.3
Endoproteinase Lys-C, sequencing grade†	—	—	5 µg	0.39
	Digestion by small amounts of proteases for 15 minutes (determination of the detection limit)‡		**Digestion by large amounts of proteases overnight (maximum of total hydrolysis)‡**	

▲ **Table 3: Limited and complete (exhaustive) digestion of casein-resorufin by different proteases.**

† available from Roche Applied Science

‡ The detection limit can be lowered by using fluorimetric analysis or by increasing the incubation time (*e.g.*, overnight).

Trademarks

Ordering Information

Protease Inhibitor Cocktails	Cat. No.	Pack Size
cOmplete Protease Inhibitor Cocktail Tablets	11 697 498 001 11 836 145 001	20 tablets 3 x 20 tablets
cOmplete, Mini Protease Inhibitor Cocktail Tablets	11 836 153 001	25 tablets
cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets	11 873 580 001	20 tablets
cOmplete, Mini, EDTA-free Protease Inhibitor Cocktail Tablets	11 836 170 001	25 tablets

Individual Protease Inhibitors	Cat. No.	Pack Size
Antipain dihydrochloride	11 004 646 001	10 mg
Aprotinin	10 236 624 001 10 981 532 001 11 583 794 001	10 mg 50 mg 100 mg
Bestatin	10 874 515 001	10 mg
Calpain Inhibitor I	11 086 090 001	25 mg
Calpain Inhibitor II	11 086 103 001	25 mg
Chymostatin	11 004 638 001	10 mg
E-64	10 874 523 001 11 585 681 001	10 mg 25 mg
Leupeptin	11 017 101 001 11 017 128 001 11 034 626 001 11 529 048 001	5 mg 25 mg 50 mg 100 mg
α_2-Macroglobulin	10 602 442 001	25 IU
Pefabloc SC	11 429 868 001 11 585 916 001 11 429 876 001	100 mg 500 mg 1 g
Pefabloc SC PLUS	11 873 601 001 11 873 628 001	set I (contains 100 mg Pefabloc SC and 5 ml PSC protector solution) set II (contains 1 g Pefabloc SC and 2 x 25 ml PSC protector solution)
Pepstatin	10 253 286 001 11 359 053 001 11 524 488 001	2 mg 10 mg 50 mg
Phosphoramidon	10 874 531 001	5 mg
PMSF	10 236 608 001 10 837 091 001 11 359 061 001	1 g 10 g 25 g
Protease Inhibitors Set	11 206 893 001	1 set
TLCK	10 874 485 001	100 mg
Trypsin Inhibitor (chicken egg white)	10 109 878 001	1 g
Trypsin Inhibitor (soybean)	10 109 886 001	50 mg

Protease Substrate	Cat. No.	Pack Size
Universal Protease Substrate (Casein, resorufin-labeled)	11 080 733 001 11 734 334 001	15 mg 40 mg

*** For more information about products for transfection, visit *www.roche-applied-science.com/transfection***

**** For additional products and information, see the 2005 Roche Applied Science Instruments and Biochemicals Catalog, or visit *www.roche-applied-science.com***

Related Products

Transfection: Achieve high transfection efficiencies and increased cell survival by using our FuGENE 6 Transfection Reagent*,**

Product	Cat. No.	Pack Size
FuGENE 6 Transfection Reagent	11 814 443 001 11 815 075 001 11 815 091 001 11 988 387 001	1 ml (300 transfections) Multi-pack (5 x 1 ml, 1,500 transfections) 0.4 ml (120 transfections) Mega-pack (5 x 1 ml, 1,500 transfections)
X-tremeGENE siRNA Transfection Reagent	04 476 093 001 04 476 115 001	1 ml (400 transfections) 5 x 1 ml (2,000 transfections)

Protein purification: Precipitate your proteins with Protein A or Protein G Agarose**

Product	Cat. No.	Pack Size
Protein A Agarose	11 719 408 001 11 134 515 001	2 ml 5 ml
Protein G Agarose	11 719 416 001 11 243 233 001	2 ml 5 ml
Immunoprecipitation Kit (Protein A)	11 719 394 001	1 kit (20 reactions)
Immunoprecipitation Kit (Protein G)	11 719 386 001	1 kit (20 reactions)

Western blotting: Detect rare proteins by using the highly sensitive Lumi-Light^{PLUS} Western Blotting Substrate**

Product	Cat. No.	Pack Size
Lumi-Light^{PLUS} Western Blotting Substrate	12 015 196 001	100 ml (1,000 cm^2 membrane)
Lumi-Light^{PLUS} Western Blotting Kit (Mouse/Rabbit)	12 015 218 001	1 kit (1,000 cm^2 membrane)
Lumi-Light Western Blotting Substrate	12 015 200 001	400 ml (4,000 cm^2 membrane)
BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit)	11 520 709 001	1 kit (2,000 cm^2 membrane)
BM Chemiluminescence Western Blotting Substrate (POD)	11 500 708 001 11 500 694 001	1 set (1,000 cm^2 membrane) 1 set (4,000 cm^2 membrane)
PVDF Western Blotting Membranes	03 010 040 001	1 roll (30 cm × 3.00 m)

Epitope tagging: Detect or purify your HA-tagged proteins by using our Anti-HA High Affinity antibody or matrix**

Product	Cat. No.	Pack Size
Anti-HA High Affinity (clone 3F10)	11 867 423 001 11 867 431 001	50 µg 500 µg
Anti-HA-Peroxidase, High Affinity	12 013 819 001	25 U (25 µg)
Anti-HA-Fluorescein, High Affinity	11 988 506 001	25 µg
Anti-HA Affinity Matrix (clone 3F10)	11 815 016 001	1.0 ml (settled resin volume)
Anti-HA (12CA5)	11 583 816 001 11 666 606 001	200 µg (lyophilized) 5 mg (1 ml)
Anti-c-myc	11 667 149 001 11 667 203 001	200 µg (Iyophilized) 5 mg (1 ml)
Anti-His_6	11 922 416 001	100 µg
Anti-His_6-Peroxidase	11 965 085 001	50 U

Reporter gene detection: Choose from a variety of products for reporter gene detection, such as our rapid and sensitive CAT ELISA**

Product	Cat. No.	Pack Size
CAT ELISA	11 363 727 001	1 kit (192 tests)
β-Gal ELISA	11 539 426 001	1 kit (192 tests)
hGH ELISA	11 585 878 001	1 kit (192 tests)
β-Gal Reporter Gene Assay, chemiluminescent	11 758 241 001	1 kit (500 assays, microplate format or 250 assays, tube format

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