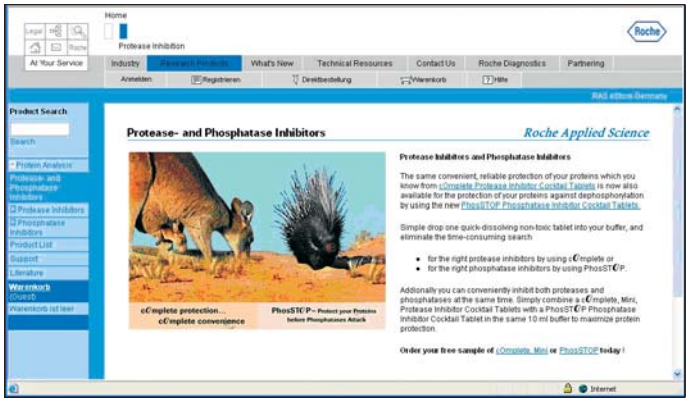
The cOmplete Guide for Protease Inhibition

cOmplete protection... cOmplete convenience

www.roche-applied-science.com

Proteases are ubiquitous in all living cells. At the time of cell lysis, proteases are released and can quickly degrade any proteins in the extract. During isolation and purification, proteolytic damage can affect funtionality and reduce yields of protein. Contaminating proteases can be inhibited by protease inhibitors, protecting the protein of interest from degradation.

Roche Applied Science offers a broad selection of protease inhibitors, as well as optimized lysis reagents, to ensure maximum yields of intact and functional proteins. The cOmplete Guide for Protease Inhibition is a resource to help you select the appropriate inhibitors and lysis reagents for your applications, and includes information regarding the specificity, stability, effectiveness, and safety of these products.



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 and Lysis Reagents

cOmplete protection... cOmplete convenience

Protein degradation due to proteases and harsh lysis conditions negatively impacts your research in many ways, leading to reduction of yields and functionality, inconsistent results, and delays in publishing.

Don't spend valuable time and money repeating experiments in order to obtain sufficient yields of intact, functional proteins. Insist on Roche Applied Science's high-quality protease inhibitors and lysis reagents to maximize success when isolating and purifying proteins.

Move your research forward by using products that provide:

Convenience – Save time with easy-to-use cOmplete Protease Inhibitor Cocktail Tablets and Lysis Reagents.

Reliability – Count on high-quality, function-tested products that have been used by researchers worldwide and referenced in thousands of publications.

Protection – Use non-toxic products that ensure maximum protein yield and functionality.

Protease Inhibitors Selection Guide

Use the following information to select the appropriate protease inhibitor for your application.

For information regarding our lysis reagents, see pages 10 through 13.

General Inhibitors for Classes of Proteases:

Serine proteases[a]	Cysteine proteases[b]	Metalloproteases[c]	Aspartic proteases[d]
PMSF		EDTA	Pepstatin
Pefabloc SC	E-64	Phosphoramidon	
Pefabloc SC PLUS		Bestatin (aminopeptidases)	
Aprotinin			
Leupeptin†			
α_2-Macroglobulin			
cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets*			
cOmplete Protease Inhibitor Cocktail Tablets*			

Protease-Specific Inhibitors:

Product:	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

High-efficiency 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

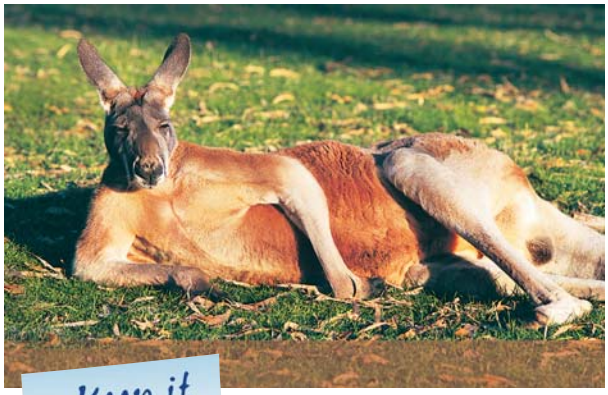
d Contain aspartic (acidic) group in the active center

† Inhibits serine and cysteine proteases with trypsin-like specificity.

* 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.

Simplify Protease Inhibition

with convenience and reliability in a cOmplete Tablet



Simplify your research with reliable products that "Keep it easy!" Save time, expense, and handling steps while increasing convenience and maximizing success.

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, quick-dissolving, water-soluble tablet. Simply add the convenient tablet to your homogenization buffer, and instantly protect your proteins against a broad range of proteases.

"…the Roche tablets were ready to use just by dissolving in the right volume of buffer. This essentially saved valuable time... I used the sample to prepare nuclear extracts from cultured transfected cells. I obtained clean and non-degraded protein samples and proteins visible by western blotting. Thanks."

— Subhagya Wadekar
UCSF

Convenience

- **Inhibit proteolytic activity in extracts from almost any tissue or cell type**, including animals, plants, yeast, bacteria, and fungi (for examples, see page 7).
- **Choose from two available tablet sizes**, with or without EDTA, for 10 or 50 ml of lysate.
- **Drop a quick-dissolving 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.
- **Benefit from multiple packaging formats –** glass vials or *EASYpacks* – for the same price.

Reliability

- **Deliver consistent doses of protease inhibition.**
- **Obtain stable, non-toxic protection** in aqueous buffers.
- **Consistently inhibit a multitude of protease classes** (see Table 1 on page 6), including serine proteases, cysteine proteases, and metalloproteases.
- **Maintain the stability of metal-dependent proteins and effectiveness of purification techniques** (*i.e.,* IMAC [immobilized metal affinity chromatography] for isolation of Poly-His-tagged proteins) by using EDTA-free cOmplete Protease Inhibitor Cocktail Tablets.

Choose our easy-to-use, versatile cOmplete Protease Inhibitor Cocktail Tablets to obtain the protection you need, with convenience and reliability. Try cOmplete Tablets today, and see how simple success can be.

Simplify Protease Inhibition

and choose the packaging that's right for you

cOmplete Protease Inhibitor Cocktail Tablets, in glass vials

Choose cOmplete Protease Inhibitor Cocktail Tablets in glass vials when protein protection in larger volumes is required. Simply dispense all twenty cOmplete Tablets from the glass vial to create one liter of protein isolation buffer.

- Rapidly dispense large quantities of cOmplete Tablets.
- Easily create large volumes of protease inhibitor solutions.

Product	Cat. No.	Pack Size
cOmplete	11 697 498 001	20 tablets
	11 836 145 001	3 x 20 tablets
cOmplete, Mini	11 836 153 001	25 tablets
cOmplete, EDTA-free	11 873 580 001	20 tablets
	05 056 489 001	3 x 20 tablets
cOmplete, Mini, EDTA-free	11 836 170 001	25 tablets

"I love the convenience, and this product would be great for people doing large scale proteomic work."

— Michael Schumacher
Johns Hopkins University

cOmplete Protease Inhibitor Cocktail Tablets, in *EASYpacks*

cOmplete Protease Inhibitor Cocktail Tablets are also available in even more convenient *EASYpacks*. Take advantage of our *EASYpacks* for all four cOmplete Tablet formats, and obtain the same reliable protease inhibition that cOmplete Tablets have always delivered.

- Easily dispense one cOmplete Tablet at a time.
- Conveniently separate the *EASYpacks* for use and storage.

***EASYpacks* allow you to pop an individually packaged tablet directly into your protein isolation buffer.**

Product	Cat. No.	Pack Size
cOmplete	04 693 116 001	20 tablets
cOmplete, Mini	04 693 124 001	30 tablets
cOmplete, EDTA-free	04 693 132 001	20 tablets
cOmplete, Mini, EDTA-free	04 693 159 001	30 tablets

Achieve cOmplete success

with convenience and reliability in a cOmplete Tablet

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.

Protect your proteins immediately after addition of cOmplete Tablets, and obtain continued protection over time.

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, 15 µg/ml	Mixture	87%	99%
Trypsin, 0.2 µg/ml	Serine	93%	89%

Table 1: Inhibition of different proteases by cOmplete Protease Inhibitor Tablets.
One cOmplete Tablet was added per 50 ml isolation buffer. Proteolytic activity was determined with our Universal Protease Substrate (casein, resorufin-labeled), Cat. No. 11 080 733 001. All experiments were performed at room temperature.

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!

Application	cOmplete Tablets	cOmplete, EDTA-free	cOmplete, Mini	cOmplete, Mini, 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 that 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.

Table 2: Choose the correct cOmplete Tablet for your application.

Achieve c𝑂mplete success

across a wide range of sample types

Achieve c𝑂mplete protection

c𝑂mplete Protease Inhibitor Cocktail Tablets have been shown to successfully inhibit protease activity across a wide range of cells, tissues, and organisms.

The following is a partial list of samples in which protease activity has been 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 Cocktail Tablets

Inhibitor	Specificity of inhibitor	Solubility/Stability
cOmplete Protease Inhibitor Cocktail Tablets (1 tablet used in 50 ml) **Supplied in glass vials:** 11 697 498 001 20 tablets 11 836 145 001 3 x 20 tablets **Supplied in *EASYpacks:*** 04 693 116 001 20 tablets	A proprietary mixture of several protease inhibitors with broad inhibitory specificity. Inhibits serine, cysteine, and metalloproteases. 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 to +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) **Supplied in glass vials:** 11 836 153 001 25 tablets **Supplied in *EASYpacks:*** 04 693 124 001 30 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 to +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) **Supplied in glass vials:** 11 873 580 001 20 tablets **Supplied in *EASYpacks:*** 04 693 132 001 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 to +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) **Supplied in glass vials:** 11 836 170 001 25 tablets **Supplied in *EASYpacks:*** 04 693 159 001 30 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 to +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.

cOmplete Cell Lysis Kits

Effectively lyse cells and protect proteins

cOmplete Lysis Kits provide ready-to-use cell Lysis Reagents along with cOmplete, Mini, Protease Inhibitor Cocktail Tablets in convenient *EASYpacks*. Simply add the cOmplete Tablets directly to the Lysis Reagent to obtain broad-spectrum protease inhibition and gentle, consistent cell lysis. Choose from cOmplete Lysis Kits optimized for bacteria, mammalian cells, and yeast cells.

Confidently protect your proteins during isolation

Obtain effective, easy cell lysis and convenient and reliable protease inhibition in the same package by choosing one of our new cOmplete Lysis products!

Convenience

- **Save time** by eliminating the need to weigh out protease inhibitors or prepare stock solutions.
- **Perform gentle cell lysis in 5–20 minutes**, without the need for traditional lysis methods such as freeze-thaw cycles, sonication, or glass beads.
- **Simply push a quick-dissolving cOmplete Tablet through the *EASYpack* foil packaging** and into 10 ml of the provided cell lysis solution.
- **Effectively inhibit a multitude of proteases at the time of cell lysis.**
- **Choose formats with or without EDTA.**

Reliability

- **Consistently lyse high percentages of cells** while protecting your proteins.
- **Obtain high yields of extracted protein** (Figure 1) by reliably inhibiting proteases and using reagents that do not denature or interact with proteins.
- **Effectively inhibit serine, cysteine, and metalloproteases.**
- **Deliver consistent doses** of protease inhibitors.

***EASYpacks* allow you to pop an individually packaged tablet directly into your lysis buffer.**

With cOmplete Lysis Kits, simply:

- Dispense the appropriate amount of Lysis Reagent
- Add a cOmplete Tablet to Lysis Reagent
- Add Lysis Reagent containing cOmplete to cells

Eliminate the need for:

- weighing and measuring buffer components
- weighing and measuring individual protease inhibitors
- preparation of multiple stock solutions
- organic solvents
- pH adjustment
- filter-sterilization
- DTT or other hazardous reagents
- multiple storage conditions
- optimization
- ordering separate components

cOmplete Cell Lysis Kits

Choose the appropriate Lysis Kit for your cell type

Lysis of Bacterial Cells

Choose the **cOmplete Lysis-B (2x) Kit** to efficiently and gently extract proteins in small volumes from bacteria (*e.g.*, *E. coli*) and simultaneously inhibit proteases in the lysate.

- **Rapidly lyse bacteria and insect cells in just 10 minutes** at room temperature.
- **Obtain protein yields that are significantly higher** than those obtained by sonication.
- **Extract soluble proteins as well as inclusion bodies** from whole bacterial lysates and insect cells infected with baculovirus.

Product	Cat. No.	Pack Size
cOmplete Lysis-B (2x)	04 719 930 001	1 kit (100 ml [2x] lysis reagent/20 tablets) for 5,000 ml culture or 20 g wet insect cell paste
cOmplete Lysis-B (2x), EDTA-free	04 719 948 001	1 kit (100 ml [2x] lysis reagent/20 tablets) for 5,000 ml culture or 20 g wet insect cell paste

Lysis of Mammalian Cells

Choose the **cOmplete Lysis-M Kit** to efficiently and gently extract proteins from both the cytoplasm and the nucleus of cultured mammalian cells, and simultaneously inhibit proteases in the lysate.

- **Efficiently lyse mammalian cells (adherent and suspension) in only 5 minutes** at room temperature.
- **Obtain protein yields that are 20–25% higher** compared to three cycles of freeze-thaw, and approximately 20% higher than two minutes of sonication (with 50% pulse).

Product	Cat. No.	Pack Size
cOmplete Lysis-M	04 719 956 001	1 kit (200 ml lysis reagent/20 tablets) for 100 g mammalian cells
cOmplete Lysis-M, EDTA-free	04 719 964 001	1 kit (200 ml lysis reagent/20 tablets) for 100 g mammalian cells

Lysis of Yeast Cells

Due to the complex and stable cell wall, yeast protein extraction is difficult and time consuming. Choose the **cOmplete Lysis-Y Kit** to efficiently and gently extract proteins from yeast cells, and simultaneously inhibit proteases in the lysate. cOmplete Lysis-Y has been successfully used to extract soluble proteins from *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, as well as a variety of gram-positive bacteria.

- **Efficiently lyse yeast cells in only 20 minutes** at room temperature.
- **Obtain protein yields that are two times higher** than those obtained by using glass beads.

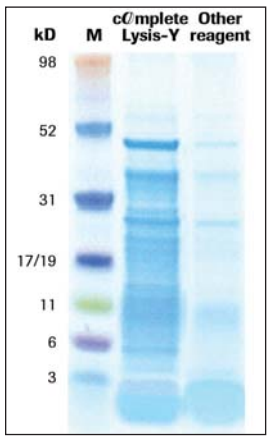


Figure 1: Comparison of yeast lysis reagents. A 130 mg sample of *Saccharomyces cerevisiae* cell pellet was resuspended in 500 µl of either cOmplete Lysis-Y reagent or the lysis reagent of another supplier. cOmplete Protease Inhibitor Cocktail Tablets were added to both preparations; the extracted whole protein fraction was analyzed by SDS-PAGE (10 µl/lane) and stained with Coomassie Blue.

Product	Cat. No.	Pack Size
cOmplete Lysis-Y	04 719 972 001	1 kit (200 ml lysis reagent/20 tablets) for 40 to 80 g wet yeast cell pellet
cOmplete Lysis-Y, EDTA-free	04 719 999 001	1 kit (200 ml lysis reagent/20 tablets) for 40 to 80 g wet yeast cell pellet

cOmplete Cell Lysis Kits

Lysis Kit	Sample type and number of reactions	Do you need an EDTA-free cOmplete Tablet?	Dissolve one tablet in	Required lysis time at room temperature
cOmplete Lysis-B (2x) 04 719 930 001 1 kit (100 ml [2x] lysis reagent/ 20 tablets)	**Bacteria and insect cells.** Lyse up to 5,000 ml of bacterial culture with an OD_{600} of 1.5 – 3.0 (approximately 20 g of wet bacterial cell paste) or up to 20 g of wet insect cell paste (or up to 400 plates of insect cell culture [100 mm]).	No	5 ml of double-concentrated Lysis-B (2x) reagent	10 min
cOmplete Lysis-B (2x), EDTA-free 04 719 948 001 1 kit (100 ml [2x] lysis reagent/ 20 tablets)	See cOmplete Lysis-B (2x) above.	Yes	5 ml of double-concentrated Lysis-B (2x) reagent	10 min
cOmplete Lysis-M 04 719 956 001 1 kit (200 ml lysis reagent/ 20 tablets)	**Mammalian cells (adherent and suspension cells).** Lyse approximately 100 g of mammalian cells.	No	10 ml of Lysis-M reagent	5 min
cOmplete Lysis-M, EDTA-free 04 719 964 001 1 kit (200 ml lysis reagent/ 20 tablets)	See cOmplete Lysis-M above.	Yes	10 ml of Lysis-M reagent	5 min
cOmplete Lysis-Y 04 719 972 001 1 kit (200 ml lysis reagent/ 20 tablets)	**Yeast cells, *E. coli*, and *Bacillus subtilis* cells.** Lyse 40 – 80 g of wet yeast cell pellet.	No	10 ml of Lysis-Y reagent	20 min
cOmplete Lysis-Y, EDTA-free 04 719 999 001 1 kit (200 ml lysis reagent/ 20 tablets)	See cOmplete Lysis-Y above.	Yes	10 ml of Lysis-Y reagent	20 min

* 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.

Volume of Lysis Reagent containing cOmplete or cOmplete, EDTA-free to add to cell pellet		Specificity of inhibitor	Notes
Bacterial culture, (OD_{600} 1.5 – 3.0)	**Volume of Lysis-B(2x) Reagent + cOmplete**	A mixture of several protease inhibitors with broad inhibitory specificity. Inhibits serine, cysteine, and metalloproteases.*	■ All inhibitors in cOmplete Tablets and the lysis reagent can be removed via dialysis. Use of a membrane with cutoff >10 kD is recommended. ■ Does not contain reducing agents such as DTT.
1.5 ml	0.15 ml		
40 ml	2.5 ml		
250 ml	5 – 10 ml		
Insect cells			
Adherent:			
100 mm plate	0.25 – 0.5 ml		
Suspension:			
1 g wet cell pellet	5 ml		
See cOmplete Lysis-B (2x) above.	See cOmplete Lysis-B (2x) above.	A mixture of several protease inhibitors with broad inhibitory specificity. Inhibits serine and cysteine proteases.*	■ 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 cOmplete Tablets and the lysis reagent can be removed via dialysis. Use of a membrane with cutoff >10 kD is recommended. ■ Does not contain reducing agents such as DTT.
Plate Size/ Surface Area	**Volume of Lysis-M Reagent + cOmplete**	See cOmplete on page 8.	■ See Notes for cOmplete Lysis-B (2x) above.
100 mm***	0.5 – 1 ml		
60 mm	0.25 – 0.5 ml		
6-well plate	0.2 – 0.4 ml per well		
24-well plate	0.1 – 0.2 ml per well		
96-well plate	0.05 – 0.1 ml per well		
See cOmplete Lysis-M above.	See cOmplete Lysis-M above.	See cOmplete, EDTA-free on page 8.	■ See Notes for cOmplete Lysis-B (2x), EDTA-free above.
Wet Yeast Cell Pellet Weight	**Volume of Lysis-Y Reagent containing cOmplete**	See cOmplete on page 8.	■ See Notes for cOmplete Lysis-B (2x) above.
50 mg	0.125 – 0.25 ml		
250 mg	0.625 – 1.25 ml		
500 mg	1.25 – 2.5 ml		
See cOmplete Lysis-Y above.	See cOmplete Lysis-Y above.	See cOmplete, EDTA-free on page 8.	■ See Notes for cOmplete Lysis-B (2x), EDTA-free above.

** 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, Mini, EDTA-free Tablets or the cOmplete Lysis kits with EDTA-free tablets.

*** Cells grown in 100 mm plates typically contain 10^7 cells (50 mg). The typical yield resulting from the extraction of 10^7 cells is approximately 3 mg of total protein.

Individual Protease Inhibitors

Obtain reliable, targeted protease inhibition

In addition to c*O*mplete Protease Inhibitor Cocktail Tablets and c*O*mplete Lysis Kits, Roche Applied Science offers a wide range of individual protease inhibitors for the isolation of intact, functional proteins. While each of our inhibitors can be used in a variety of applications, they share common advantages:

- **Rely on high-purity protease inhibitors** that are free of contaminating proteases that could damage your protein sample.
- **Count on inhibitors that are function-tested** to ensure that each lot delivers consistent, reliable protection of your proteins.
- **Choose individual inhibitors** to meet different application needs.
- **Create combinations of inhibitors** for specific application requirements.
- **Select products to inhibit serine proteases, metalloproteases, acid proteases, and more.**

Related Products

Perform easy, economical screening for the correct inhibitor for your application by using the **Protease Inhibitors Set**, which includes ten different individually packaged inhibitors.

Product	Cat. No.	Pack Size
Protease Inhibitors Set	11 206 893 001	1 set

Verify protease inhibition by using the **Universal Protease Substrate** to detect trace quantities of proteolytic activity in less than one hour.

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

*Patent 0209875 and US 4.954.630 owned by Roche Diagnostics GmbH.

Pefabloc SC and Pefabloc SC PLUS

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 fluoro-phosphate) were used to eliminate this problem.

Despite the popularity of PMSF and DFP, both have serious disadvantages. Benefit from a safe, effective alternative: choose **Pefabloc SC** 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. PMSF and DFP, however, 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 effective protease inhibition without risk to you or those around you.

Reliability

- **Ensure protection with improved stability –** Pefabloc SC remains highly active in aqueous solutions, even at pH levels above 7.0 and temperatures above 4°C, protecting your proteins long after PMSF and DFP have failed (Figure 2).
- **Maximize inhibition –** Superior solubility and stability 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.

Sulfonyl-type serine protease inhibitors such as Pefabloc SC and PMSF can bind covalently to proteins. 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 with Pefabloc SC PLUS

- **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 with Pefabloc SC PLUS

- **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).

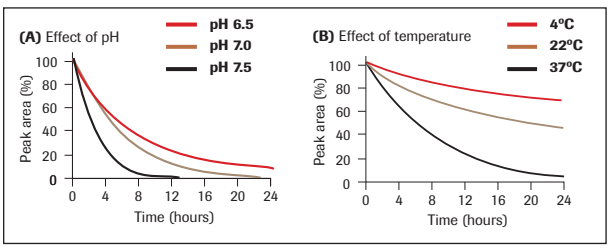


Figure 2: The effect of temperature and pH on the stability of Pefabloc SC. Graph (A) shows the relative stability of Pefabloc SC (5.0 mg/ml) in an aqueous phosphate buffer at pH 7.0. Graph (B) shows the relative stability of Pefabloc SC (5.0 mg/ml) in an aqueous phosphate buffer at 37°C.

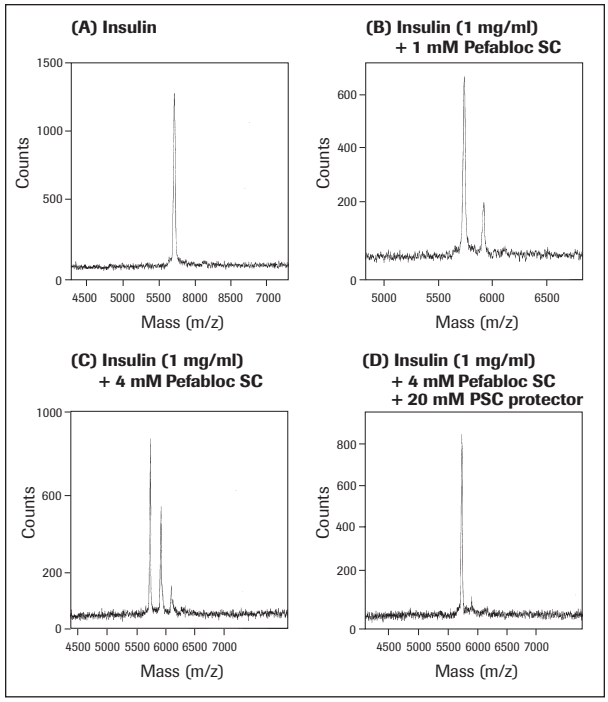


Figure 3 (A–D): Mass spectrograms showing the covalent interaction between insulin and the protease inhibitor Pefabloc SC (PSC). Diagram A is the insulin blank. At 1 mM Pefabloc SC, the formation of the binding is visible as a second peak formation (Diagram B). Higher concentrations of the protease inhibitor 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.

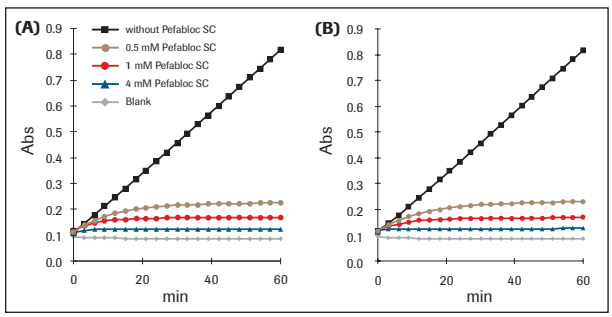


Figure 4: Chymotrypsin inhibition with Pefabloc SC in the absence (A) and presence (B) of the Pefabloc SC protector. Results show no change in the effectiveness of inhibition.

Individual Protease Inhibitors

Inhibitor	Specificity of inhibitor	Suggested starting concentration**	Mode of action of inhibitor
Antipain dihydrochloride (Papain Inhibitor) 11 004 646 001 10 mg	Inhibits papain and trypsin. Plasmin is inhibited to a small extent.	50 µg/ml (74 µM) (1 U of papain is inhibited to 49% by 0.9 µg of antipain.)	Reversible
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.	0.06–2.0 µg/ml (0.01–0.3 µM)	Reversible
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.	40 µg/ml (130 µM)	Reversible
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.	17 µg/ml	Reversible
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.	7 µg/ml	Reversible
Chymostatin 11 004 638 001 10 mg	Specific inhibitor of α-, β-, γ-, δ-chymotrypsin.	6–60 µg/ml (10–100 µM) Unit definition: One unit chymotrypsin is inhibited to 49% by 1.8 µg of chymostatin.	Reversible
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 such as cathepsin B and L.	0.5–10 µg/ml (1.4–28.0 µM)	Irreversible
Leupeptin Ac-Leu-Leu-argininal 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.	0.5 µg/ml (1 µM)	Irreversible

* 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.*

Solubility/Stability*	Notes
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.	■ 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.
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 to +8°C. Aliquots stored at –15 to –25°C are stable for approximately 6 months.	■ Molecular Weight: 6,512 ■ Avoid repeated freeze-thaws and exposure to strong alkali solutions. ■ Aprotinin is inactive at pH >12.8.
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.	■ 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*.
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 to +8°C and approximately 4 weeks at –15 to –25°C. We recommend making solutions up fresh before use.	■ Molecular Weight: 383.5 ■ Not soluble in water.
(See Calpain Inhibitor I, above).	■ Molecular Weight: 401.6 ■ Not soluble in water.
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.	■ Molecular Weight: 607.71
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.	■ Molecular Weight: 357.4 ■ Stable between pH 2–10. ■ Unstable in strong alkali and strong mineral acids.
Highly soluble in water (1 mg/ml). Stable for at least 1 week at +2 to +8°C and 6 months frozen in aliquots at –15 to –25°C.	■ 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	Suggested starting concentration**	Mode of action of inhibitor
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).	Unit definition: One inhibitor unit inhibits 9.1 µg of trypsin.	Reversible
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.	0.1–1.0 mg/ml (0.4–4 mM)	Irreversible
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.	0.1–1.0 mg/ml (0.4–4.0 mM)	Irreversible
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.	0.7 µg/ml (1 µM)	Reversible
Phosphoramidon **N-(α-Rhamnopyra-nosyloxy-hydroxyphosphinyl)-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).*	4–330 µg/ml (7–570 µM)	Irreversible
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).	17–170 µg/ml (0.1–1 mM)	Irreversible
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.	37–50 µg/ml (100–135 µM)	Irreversible
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.	10–100 µg/ml	Reversible

* 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.*

Solubility/Stability*	Notes
Soluble in water. Stable for at least 1 week at room temperature or 3 weeks at +2 to +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.	■ 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.
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).	■ Molecular Weight: 239.5 ■ A safe, stable, and water-soluble alternative to PMSF and DFP.
Solubility and stability of the protease inhibitor remains unchanged. See Pefabloc SC.	■ Sets contain Pefabloc SC (PSC) and a special protector (PSC protector). ■ The set eliminates interaction between Pefabloc SC and sample proteins.
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 to +8°C, or 1 month if stored in aliquots at –15 to –25°C.	■ Molecular Weight: 685.9 ■ Insoluble in water.
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.	■ Molecular Weight: 579.6
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 +15 to +25°C.	■ 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.
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).	■ 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.
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.	■ 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.

Trademarks

Roche Diagnostics GmbH
Roche Applied Science
68296 Mannheim
Germany

04854896990 • 08/07

www.roche-applied-science.com