

# cOmplete, EDTA-free

## Protease Inhibitor Cocktail Tablets provided in *EASYpack*

For the complete inhibition of serine and cysteine proteases during extractions from animal and plant tissues or cells, yeast and bacteria

**Cat. No. 04 693 132 001**

20 tablets

 **Do not eat**

 **Version 03**

Content version: December 2005

Store at +2 to +8°C

## 1. What this Product Does

### 1.1 Properties

cOmplete, EDTA-free tablets inhibit a broad spectrum of serine and cysteine proteases. In contrast to other cOmplete tablets they do not contain EDTA leaving the stability and the function of metal dependant proteins unaffected. The affinity purification of Poly-His tagged fusionproteins via IMAC (immobilized metal affinity chromatography) is also facilitated (no dialysis necessary).

Due to the optimized composition of the tablets they show excellent inhibition of serine and cysteine proteases and are therefore very well suited for the protection of proteins isolated from animal tissues, plants, yeast and bacteria. cOmplete, EDTA-free tablets contain both irreversible and reversible protease inhibitors. Metallo and aspartic proteases are not inhibited.

### 1.2 Contents

20 individually packed cOmplete Protease Inhibitor Cocktail Tablets in foil blisters. Each tablet is sufficient for a volume of 50 ml solution.

### 1.3 Stability

- The tablets are stable at +2 to +8°C, stored dry, until expiration date.
- The stock solution is stable for 1–2 weeks, stored at +2 to +8°C, or at least for 12 weeks at –15 to –25° C.

### 1.4 Application

Used for the inhibition of serine and cysteine proteases in bacterial, yeast, plant and animal cell extracts.

For the inhibition of proteolytic activity in larger volumes, in which EDTA may interfere with protein stability or subsequent assays or purification methods (e.g., Immobilized Metal Chelate Affinity Chromatography [IMAC]).

## 2. How to Use this Product

### 2.1 Handling

Carefully push the tablet through the foil packaging using the base of your thumb (and not your finger nail) to prevent the breakage of tablets.

 **Do not eat the tablets.**

### 2.2 Preparation of Working Solutions

One cOmplete, EDTA-free tablet is sufficient for the inhibition of the proteolytic activity in 50 ml extraction solution. If very high proteolytic activity is present, one tablet should be used for 25 ml extraction buffer. The tablets can be added directly to the extraction medium. Alternatively a stock solution (25 × conc.) can be prepared.

If it is necessary to inhibit proteolytic activity in a smaller volume we recommend to use cOmplete, Mini, EDTA-free tablets (1 tablet for 10 ml extraction solution, see "Ordering Information").

### 2.3 Stock solution (25 × conc.)

Dissolve one cOmplete, EDTA-free tablet in 2 ml redist. H<sub>2</sub>O or in 100 mM phosphate buffer, pH 7.0.

## 3. Results

cOmplete, EDTA-free tablets are applied to stabilize those extracts where the stability or activity of metal containing proteins should not be effected. Since EDTA interferes with IMAC (immobilized metal affinity chromatography) cOmplete, EDTA-free tablets are preferentially used in the isolation process of Poly-His tagged fusion proteins. cOmplete, EDTA-free tablets inhibit efficiently serine and cysteine proteases in a broad range but not metalloproteases.

Occasionally, aspartic proteases ("acid proteases") can interfere upon isolations from animal tissues or bacterial extracts (1). These proteases, however, exhibit pronounced activities only at low pH values. If extraction or single isolation steps have to be performed at this pH range the addition of pepstatin\* is recommended to inhibit aspartic protease activity as a precaution.

Typical values for the inhibition of different proteases and protease mixtures by cOmplete, EDTA-free tablets are shown in table 1.

Protease resp. protease mixture	Enzyme concentration (mg/ml)	pH-value	% inhibition after immediate addition to the protease	% inhibition after 60 min incubation (protease + cOmplete) at +15 to +25° C
Pancreas-extract	0.02	7.8	84%	94%
Thermolysin (Metalloprotease)	0.0005	7.8	7%	10%
Chymotrypsin	0.002 0.02	7.8 7.8	90% 60%	100% 98%
Trypsin	0.02 0.002	7.8 7.8	70% 81%	74% 86%
Papain	0.33	7.8	74%	87%

**Table 1: Inhibition of different proteases by cOmplete Protease Inhibitor Tablets.**

One cOmplete, EDTA-free tablet was added per 50 ml incubation solution. Proteolytic activity was determined with the Roche Applied Science Universal Protease Substrate (casein, resorufin-labeled\*). When extractions or single-step isolations are necessary in the acid pH range, simply include pepstatin\* along with cOmplete, EDTA-free tablets to ensure aspartic (acid) protease inhibition. All experiments were performed at +15 to +25°C.

\* available from Roche Applied Science

## 4. Additional Information on this Product

### 4.1 Absorption

A solution of one cOmplete, EDTA-free tablet dissolved in 50 ml H<sub>2</sub>O has an absorption of 0.08 at 280 nm.

### 4.2 Quality Control

The inhibitory activity of each lot is tested with a concentrated pancreas extract. Thereby, the proteolytic activity is typically inhibited by 94% after one hour (detection with universal protease substrate, casein, resorufin-labeled\*).

### 4.3 Reference

- 1 North, M.J. (1969) in: Proteolytic Enzymes - A Practical Approach (Beynon, P.J. & Bond, J.S. eds.), IRL press Oxford, pp. 117-119.

### 4.4 Changes to previous version

- Regulatory Disclaimer updated
- Editorial changes

## 5. Ordering Information

For additional information, please visit  
[www.roche-applied-science.com/proteaseinhibitor](http://www.roche-applied-science.com/proteaseinhibitor)

### cOmplete Protease Inhibitor Cocktail Tablets in EASYpacks:

Product	Pack size	Cat. No.
cOmplete	20 tablets in foil blisters (for 50 ml each)	04 693 116 001
cOmplete, Mini	30 tablets in foil blisters (for 10 ml each)	04 693 124 001
cOmplete, EDTA-free	20 tablets in foil blisters (for 50 ml each)	04 693 132 001
cOmplete, Mini, EDTA-free	30 tablets in foil blisters (for 10 ml each)	04 693 159 001

### cOmplete Protease Inhibitor Cocktail Tablets in glass vials:

Product	Pack size	Cat. No.
cOmplete	20 tablets in a glass vial (for 50 ml each)	11 697 498 001
	3 × 20 tablets in a glass vial (for 50 ml each)	11 836 145 001
cOmplete, Mini	25 tablets in a glass vial (for 10 ml each)	11 836 153 001
cOmplete, EDTA-free	20 tablets in a glass vial (for 50 ml each)	11 873 580 001
cOmplete, Mini, EDTA-free	25 tablets in a glass vial (for 10 ml each)	11 836 170 001

### Kits and Sets:

Product	Pack size	Cat. No.
Pefabloc SC PLUS	Set I: contains 100 mg Pefabloc SC and 5 ml PSC protector solution	11 873 601 001
	Set II: contains 1g Pefabloc SC and 2 × 25 ml PSC protector solution	11 873 628 001
Protease Inhibitor Set	Small quantities of 10 most commonly used protease inhibitors	11 206 893 001

### Substrate:

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

### Individual Protease Inhibitors:

Product	Pack size	Cat. No.
Aprotinin	10 mg	10 236 624 001
	50 mg	10 981 532 001
	100 mg	11 583 794 001
Bestatin	10 mg	10 874 515 001
	50 mg	11 359 070 001
Calpain Inhibitor I	25 mg	11 086 090 001
Calpain Inhibitor II	25 mg	11 086 103 001
Chymostatin	10 mg	11 004 638 001
E-64	5 mg	11 585 673 001
	10 mg	10 874 523 001
	25 mg	11 585 681 001
Leupeptin	5 mg	11 017 101 001
	25 mg	11 017 128 001
	50 mg	11 034 626 001
	100 mg	11 529 048 001
α <sub>2</sub> -Macroglobulin	25 IU	10 602 442 001
Pefabloc SC	100 mg	11 429 868 001
	500 mg	11 585 916 001
	1 g	11 429 876 001
Pepstatin	2 mg	10 253 286 001
	10 mg	11 359 053 001
	50 mg	11 524 488 001
PMSF	1 g	10 236 608 001
	10 g	10 837 091 001
	25 g	11 359 061 001
TLCK - HCl	100 mg	10 874 485 001
	250 mg	10 874 493 001
Trypsin Inhibitor (chicken, egg white)	1 g	10 109 878 001
Trypsin Inhibitor (soybean)	50 mg	10 109 886 001
	500 mg	10 109 894 001

This combination comprises only the most important products related to the product described.

Please refer to our latest catalog for our current product range or contact your local Roche Applied Science representative directly.

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### Regulatory Disclaimer

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