

# c@mplete

# **Protease Inhibitor Cocktail Tablets in glass vials**

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

**Cat. No. 11 697 498 001** 20 tablets **Cat. No. 11 836 145 001** 3 × 20 tablets

**!** Version 12.0

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Store at +2 to +8°C

#### 1. What this Product Does

#### 1.1 Properties

c@mplete tablets inhibit a broad spectrum of serine, cysteine and metalloproteases as well as calpains. Due to the optimized composition of the tablets they show excellently inhibition effects and are therefore very well suited for the protection of proteins isolated from animal tissues, plants, yeast and bacteria. c@mplete contains both irreversible and reversible protease inhibitors.

#### 1.2 Contents

20 (or  $3\times 20$ , resp.) c@mplete Protease Inhibitor Cocktail Tablets in a glass vial. 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, cysteine, and metalloproteases in bacterial, mammalian, yeast, and plant cell extracts.

c@mplete contains both reversible and irreversible protease inhibitors. Therefore, we recommend the addition of c@mplete to all stock buffers and solutions normally protected with protease inhibitors and not only during the initial purification steps.

c € mplete contains EDTA (18.5 mg/tablet yield 1 mM solution of EDTA in 50 ml). Therefore, the extraction buffer should not contain divalent cations like Ca²+, Mg²+ or Mn²+ otherwise the inhibition of the metalloproteases might be incomplete. If the protein of interest will be purified by IMAC (immobilized metal- chelate affinity chromatography), *e.g.* Poly-His tagged recombinant proteins, EDTA has to be eliminated (*e.g.* by dialysis) prior to the chromatography.

Alternatively, the product  $c\mathcal{O}$ mplete, EDTA-free can be used (see table "Ordering Information"). These tablets are identical to  $c\mathcal{O}$ mplete, with the only difference that no EDTA or other chelating agent are present.

# 2. How to Use this Product

# 2.1 Preparation of Working Solutions

One tablet  $c\mathcal{Q}$  mplete 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  $\times$  conc.) can be prepared.

If it is necessary to inhibit proteolytic activity in a smaller volume we recommend to use  $c\mathcal{O}$ mplete, Mini (1 tablet for 10 ml extraction solution, see "Ordering Information"). The composition of the  $c\mathcal{O}$ mplete Mini tablet is identical to the normal  $c\mathcal{O}$ mplete tablet, therefore comparable results are achieved with both product types.

#### 2.2 Stock solution (25 $\times$ conc.)

Dissolve one tablet  $c\mathcal{O}$ mplete in 2 ml redist.  $H_2O$  or in 100 mM phosphate buffer, pH 7.0.

#### 3. Results

In extracts from animal tissues mainly serine, cysteine and metalloproteases are found; in plant extracts serine and cysteine proteases dominate. For bacterial extracts serine and metalloproteases are typical (1). c $\mathcal{O}$ mplete tablets inhibit efficiently serine, cysteine and metalloproteases in a broad range.

Occasionally, aspartic proteases ("acid proteases") can interfere upon isolations from animal tissues. 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  $c\mathcal{O}$  mplete are shown in table 1.

Protease resp. protease mixture	Enzyme concen- tration (mg/ml)	pH- value	% inhibition after immediate addition to the protease	% inhibition after 60 min incubation (protease + c@mplete) at +15 to +25° C
Pancreas- extract	0.015 0.03	7.8 6.5	87% 88%	99% 96%
Pronase	0.0015 0.003	7.8 7.0	88% 90%	99% 95%
Thermolysin	0.0008	7.8	99%	100%
Chymotrypsin	0.0015	7.8	97%	97%
Trypsin	0.0002	7.8	93%	89%
Papain	1.0	6.5	95%	73%

Table 1: Inhibition of different proteases by c O mplete Protease Inhibitor Tablets.

One c\$\mathcal{O}\text{mplete}\$ 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 c\$\mathcal{O}\text{mplete}\$ tablets to ensure aspartic (acid) protease inhibition. All experiments were performed at room temperature.

Personal research communications indicate that acetylcholinesterase is strongly inhibited by c@mplete. Butyrylcholin-esterase is inhibited to a lesser extent.

<sup>\*</sup> available from Roche Applied Science

# 4. Additional Information on this Product

#### 4.1 Absorption

A solution of 1 tablet c ${\cal O}$ mplete dissolved in 50 ml  ${\rm H_2O}$  has an absorption of 0.08 at 280 nm.

#### 4.2 Quality Control

The inhibitory power of c 

mplete has been demonstrated with many proteases and protease mixtures. In these experiments drastically higher concentrations of proteases were used compared to the concentration usually present in extracts (see table 1). The inhibitory activity of each lot is tested with a concentrated pancreas extract and a concentrated pronase solution. Thereby, the proteolytic activities are typically inhibited by 95% 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.

# 5. Ordering Information

For addional information, please visit www.roche-applied-science.com/proteaseinhibitor

## c@mplete Protease Inhibitor Cocktail Tablets in glass vials:

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

# c@mplete Protease Inhibitor Cocktail Tablets in EASYpacks:

Product	Pack size	Cat. No.
c <b>Ø</b> mplete	20 tablets in foil blisters (for 50 ml each)	04 693 116 001
c <b>Ø</b> mplete, Mini	30 tablets in foil blisters (for 10 ml each)	04 693 124 001
c <b>Ø</b> mplete, EDTA-free	20 tablets in foil blisters (for 50 ml each)	04 693 132 001
c <b>Ø</b> mplete, Mini, EDTA- free	30 tablets in foil blisters (for 10 ml each)	04 693 159 001

# Kits and Sets:

Product	Pack size	Cat. No.
Pefabloc SC PLUS	Set I: contains 100 mg Pefabloc SC and 5 ml PSC protector solution Set II: contains 1g Pefabloc SC and 2 × 25 ml PSC protector solution	11 873 601 001 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 40 mg	11 080 733 001 11 734 334 001

#### **Individual Protease Inhibitors:**

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

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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