For life science research only. Not for use in diagnostic procedures.



# **cOmplete, Protease Inhibitor Cocktail Tablets provided in** *EASYpack*

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

**Cat. No. 04 693 116 001** 20 tablets

for 50 ml each

Store product at +2 to +8°C.

1.	General Information	3
1.1.	Contents	3
1.2.	Storage and Stability	3
	Storage Conditions (Product)	
	Storage Conditions (Working Solution)	
1.3.	Additional Equipment and Reagent required	3
1.4.	Application	3
2.	How to Use this Product	4
2.1.	Before you Begin	4
	Safety Information	
	Working Solution	
	Preparation of 25x-concentrated stock solution	
2.2.	Parameters	
	Absorption	
	Working Concentration	
3.	Results	5
4.	Additional Information on this Product	5
4.1.	Quality Control	5
5.	Supplementary Information	6
5.1.	Conventions	6
5.2.	Changes to previous version	6
5.3.	Ordering Information	6
5.4.	Trademarks	7
5.5.	License Disclaimer	7
5.6.	Regulatory Disclaimer	7
5.7.	Safety Data Sheet	7
5.8.	Contact and Support	7

#### 1. General Information

#### 1.1. Contents

Package	Label	Function / Description	Content
1	cOmplete, Protease inhibitor cocktail tablets	<ul><li>Individually packaged tablets.</li><li>Each tablet is sufficient for a volume of 50 ml solution.</li></ul>	20 foil blister packs, 1 tablet each

## 1.2. Storage and Stability

#### **Storage Conditions (Product)**

When stored at +2 to +8°C, the product is stable through the expiration date printed on the label.

Package	Label	Storage
1	cOmplete,	Store dry at +2 to +8°C.
	Protease inhibitor cocktail tablets	

#### **Storage Conditions (Working Solution)**

Store stock solution at +2 to +8°C for 1 to 2 weeks or -15 to -25°C for at least 12 weeks.

## 1.3. Additional Equipment and Reagent required

#### For preparation of stock solution

Double-distilled water or 100 mM phosphate buffer, pH 7.0

#### For extraction in the acid pH range

Pepstatin\*

#### For determination of proteolytic activity

Universal Protease Substrate, Casein, resorufin-labeled\*

## 1.4. Application

cOmplete Protease inhibitor cocktail tablets are used for the inhibition of a broad spectrum of serine, cysteine, and metalloproteases, as well as calpains. Due to the optimized composition of the tablets, they show excellent inhibition effects and are well suited for the protection of proteins isolated from:

- Mammalian tissue
- Bacteria
- Yeast
- Plants

cOmplete contains both reversible and irreversible protease inhibitors, therefore, add cOmplete to all stock buffers and solutions normally protected with protease inhibitors, and not only during the initial purification steps. Since cOmplete contains EDTA (18.5 mg/tablet yields a 1 mM solution of EDTA in 50 ml), the extraction buffer should not contain divalent cations such as 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 Affinity Chromatography), for example, poly-His tagged recombinant proteins, EDTA must be eliminated, for example, by dialysis prior to the chromatography.

Alternatively, the product cOmplete, EDTA-free\* can be used. These tablets are identical to cOmplete, with the only difference that no EDTA or other chelating agents are present.

#### 2. How to Use this Product

#### 2.1. Before you Begin

#### **Safety Information**

Do not eat the tablets.

#### **Working Solution**

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

One cOmplete Protease inhibitor tablet is sufficient for the inhibition of the proteolytic activity in 50 ml extraction solution. If very high proteolytic activity is present, use one tablet for 25 ml extraction buffer. Tablets can be added directly to the extraction medium. Alternatively, a 25x-concentrated stock solution can be prepared.

If it is necessary to inhibit proteolytic activity in a smaller volume, use cOmplete, Mini\* (1 tablet for 10 ml extraction solution). The composition of the cOmplete Mini tablet is identical to the normal cOmplete tablet, therefore comparable results are achieved with both products.

#### **Preparation of 25x-concentrated stock solution**

Dissolve one cOmplete Protease inhibitor tablet in 2 ml double-distilled water or in 2 ml 100 mM phosphate buffer, pH 7.0.

#### 2.2. Parameters

## **Absorption**

A solution of 1 tablet cOmplete inhibitor dissolved in 50 ml water has an absorption of 0.08 at 280 nm.

## **Working Concentration**

1 tablet per 50 ml extraction solution.

#### 3. Results

cOmplete tablets efficiently inhibit serine, cysteine, and metalloproteases in a broad range.

Extract from	Type of protease present
Animal tissues	Serine, cysteine, and metalloproteases
Plants	Serine and cysteine proteases
Bacterial	Serine and metalloproteases

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 must be performed at this pH range, add Pepstatin\* to inhibit aspartic protease activity as a precaution.

Typical values for the inhibition of different proteases and protease mixtures by cOmplete are shown in the following table. One cOmplete tablet was added per 50 ml incubation solution. Proteolytic activity was determined with the Universal Protease Substrate, Casein, resorufin-labeled\*. When extractions or single-step isolations are necessary in the acid pH range, include Pepstatin\* along with cOmplete tablets to ensure aspartic (acid) protease inhibition. All experiments were performed at +15 to +25°C.

is inhibited to a lesser extent.

Protease respective protease mixture	Enzyme concentration [mg/ml]	pH value	% Inhibition after immediate addition to the protease	% Inhibition after no 1 hour incubation with the protease
Pancreas extract	0.015	7.8	87	99
	0.03	6.5	88	96
Pronase	0.0015	7.8	88	99
	0.003	7.0	90	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

The inhibitory power of cOmplete 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 above.

## 4. Additional Information on this Product

## 4.1. Quality Control

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\*).

# 5. Supplementary Information

#### 5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols			
information Note: Additional information about the current topic or procedure.			
⚠ Important Note: Information critical to the success of the current procedure or use of the product.			
1 2 3 etc.	Stages in a process that usually occur in the order listed.		
1 2 3 etc.	Steps in a procedure that must be performed in the order listed.		
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.		

# 5.2. Changes to previous version

Layout changes. Editorial changes.

# **5.3. Ordering Information**

Product	Pack Size	Cat. No.	
Reagents, kits			
cOmplete, Mini	25 tablets in a glass vial, for 10 ml each	11 836 153 001	
Universal Protease Substrate	40 mg	11 734 334 001	
Pepstatin	2 mg	10 253 286 001	
	10 mg	11 359 053 001	
	50 mg	11 524 488 001	
cOmplete, EDTA-free	20 tablets in a glass vial, for 50 ml each	11 873 580 001	
	3 x 20 tablets in glass vials, for 50 ml each	05 056 489 001	

#### 5.4. Trademarks

All product names and trademarks are the property of their respective owners.

#### 5.5. License Disclaimer

For patent license limitations for individual products please refer to: **List of biochemical reagent products**.

#### 5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

## **5.7. Safety Data Sheet**

Please follow the instructions in the Safety Data Sheet (SDS).

## 5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.