

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



cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets provided in *EASYpack*

 **Version: 06**

Content Version: March 2021

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
for 50 ml each

Store product at +2 to +8°C.

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1. General Information

1.1. Contents

Package	Label	Function / Description	Content
1	cOplete, EDTA-free, Protease inhibitor cocktail tablets	<ul style="list-style-type: none"> Individually packaged tablets. Each tablet is sufficient for a volume of 50 ml solution. 	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	cOplete, EDTA-free, Protease inhibitor cocktail tablets	Store dry at +2 to +8°C.

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 protection of phosphatases

- PhosSTOP, Phosphatase inhibitor cocktail tablets in EASYpacks*

For determination of proteolytic activity

- Universal Protease Substrate, Casein, resorufin-labeled*

1.4. Application

cOplete, EDTA-free, Protease inhibitor cocktail tablets are used for the inhibition of a broad spectrum of serine and cysteine proteases. The inhibitor is ideal for the inhibition of proteolytic activity in larger volumes, in which EDTA may interfere with protein stability or subsequent assays or purification methods, such as IMAC (Immobilized Metal Affinity Chromatography). 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

cOplete, EDTA-free contains both irreversible and reversible protease inhibitors. In contrast to other cOplete tablets, they do not contain EDTA, leaving the stability and the function of metal-dependent proteins unaffected. The affinity purification of Poly-His tagged fusion proteins via IMAC is also facilitated (no dialysis necessary).

i *Metalloproteases and aspartic proteases are not inhibited.*

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 cOplete EDTA-free 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 cOplete, Mini, EDTA-free* (1 tablet for 10 ml extraction solution). The composition of the cOplete, Mini, EDTA-free tablet is identical to the normal cOplete, EDTA-free tablet, therefore, comparable results are achieved with both products.

Preparation of 25x-concentrated stock solution

Dissolve one cOplete, EDTA-free 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 cOplete, EDTA-free 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, EDTA-free tablets are applied to stabilize those extracts where the stability or activity of metal-containing proteins should not be affected. Since EDTA interferes with IMAC, cOmplete, EDTA-free is 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 in isolations from animal tissues or bacterial extracts. 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.

To protect proteins from both proteases and phosphatases, combine cOmplete Protease inhibitor cocktail tablets with PhosSTOP Phosphatase inhibitor cocktail tablets*.

Typical values for the inhibition of different proteases and protease mixtures by cOmplete, EDTA-free are shown in the following table. One cOmplete, EDTA-free 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, EDTA-free tablets to ensure aspartic (acid) protease inhibition. All experiments were performed at +15 to +25°C.

Protease respective protease mixture	Enzyme concentration [mg/ml]	pH value	% Inhibition after immediate addition to the protease	% Inhibition after 1 hour incubation with the protease
Pancreas extract	0.02	7.8	84	94
Thermolysin (Metalloprotease)	0.0005	7.8	7	10
Chymotrypsin	0.002	7.8	90	100
	0.02	7.8	60	98
Trypsin	0.02	7.8	70	74
	0.002	7.8	81	86
Papain	0.33	7.8	74	87

4. Additional Information on this Product









4.1. Quality Control

The inhibitory activity of each lot is tested with a concentrated pancreas extract. Thereby, the proteolytic activities are typically inhibited by 94% 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	
 <i>Information Note: Additional information about the current topic or procedure.</i>	
 Important Note: Information critical to the success of the current procedure or use of the product.	
   etc.	Stages in a process that usually occur in the order listed.
   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		
PhosSTOP	10 tablets, for 10 ml each	04 906 845 001
	20 tablets, for 10 ml each	04 906 837 001
cOplete, Mini, EDTA-free	25 tablets in a glass vial, for 10 ml each	11 836 170 001
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

5.4. Trademarks

PHOSSTOP is a trademark of Roche.

All other 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.

