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Not for use in diagnostic procedures.



cOmplete, Mini Protease Inhibitor Cocktail Tablets provided in a glass vial

 **Version: 12**

Content Version: November 2020

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

Cat. No. 11 836 153 001 25 tablets in a glass vial
for 10 ml each

Store product at +2 to +8°C.

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1. General Information

1.1. Contents

| Vial / Bottle | Label | Function / Description | Content |
|---------------|---|---|-----------------------------|
| 1 | cOplete, Mini, Protease inhibitor cocktail tablets | Each tablet is sufficient for a volume of 10 ml solution. | 1 glass vial, 25 tablets |

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.

| Vial / Bottle | Label | Storage |
|---------------|---|--------------------------|
| 1 | cOplete, Mini, 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 determination of proteolytic activity

- Universal Protease Substrate, Casein, resorufin-labeled*

1.4. Application

cOplete, Mini 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

cOplete, Mini contains both reversible and irreversible protease inhibitors, therefore add cOplete, Mini to all stock buffers and solutions normally protected with protease inhibitors and not only during the initial purification steps.

Since cOplete, Mini contains EDTA (3.7 mg/tablet yields a 1 mM solution of EDTA in 10 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 cOplete, Mini, EDTA-free* can be used. These tablets are identical to cOplete, Mini, 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.

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

One cOplete, Mini Protease inhibitor tablet is sufficient for the inhibition of the proteolytic activity in 10 ml extraction solution. If very high proteolytic activity is present, use one tablet for 7 ml extraction buffer. Tablets can be added directly to the extraction medium. Alternatively, a 7x-concentrated stock solution can be prepared.

If it is necessary to inhibit proteolytic activity in a larger volume, use cOplete* (1 tablet for 50 ml extraction solution). The composition of the cOplete tablet is identical to the normal cOplete, Mini tablet, therefore comparable results are achieved with both products.

Preparation of 7x-concentrated stock solution

Dissolve one cOplete, Mini Protease inhibitor tablet in 1.5 ml double-distilled water or in 1.5 ml 100 mM phosphate buffer, pH 7.0.

2.2. Parameters

Absorption

A solution of 1 tablet cOplete, Mini inhibitor dissolved in 10 ml water has an absorption of 0.08 at 280 nm.

Working Concentration

1 tablet per 10 ml extraction solution.

3. Results

cOplete, Mini 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 in 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 cOplete, Mini are shown in the following table. One cOplete, Mini tablet was added per 10 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 cOplete, Mini 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 | 85 | 96 |
| Pronase | 0.005 | 7.8 | 92 | 98 |
| Thermolysin | 0.0005 | 7.8 | 98 | 98 |
| Chymotrypsin | 0.0015 | 7.8 | 85 | 93 |
| | 0.003 | 7.8 | 90 | 93 |
| Papain | 0.33 | 7.8 | 66 | 77 |

The inhibitory power of cOplete, Mini 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

For lot-specific certificates of analysis, see section **Contact and Support**.

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>i</i> | 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.

Update to include new safety Information to ensure handling according controlled conditions.

5.3. Ordering Information

| Product | Pack Size | Cat. No. |
|------------------------------|---|----------------|
| Reagents, kits | | |
| cOplete | 20 tablets in a glass vial, for 50 ml each | 11 697 498 001 |
| | 3 x 20 tablets in glass vials, for 50 ml each | 11 836 145 001 |
| 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

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.

