

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



# Pefabloc<sup>®</sup> SC PLUS

## 4-(2-Aminoethyl)-benzenesulfonyl fluoride hydrochloride

 **Version: 09**

Content Version: November 2020

|                                |  |
|--------------------------------|--|
| <b>Cat. No. 11 873 601 001</b> | Set I<br>100 mg Pefabloc <sup>®</sup> SC; 5 ml PSC-Protector solution    |
| <b>Cat. No. 11 873 628 001</b> | Set II<br>1 g Pefabloc <sup>®</sup> SC; 2 x 25 ml PSC-Protector solution |

**Store the product at +2 to +8°C.**

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# 1. General Information

## 1.1. Contents

| Vial / Bottle | Cap   | Label  | Function / Description                       | Catalog Number | Content                 |
|---------------|-------|--|--|----------------|-------------------------|
| <b>Set 1</b>  |       |  |  |                |                         |
| 1             | black | Pefabloc SC <sup>®</sup> PLUS,<br>Pefabloc SC <sup>®</sup> | White powder                                 | 11 873 601 001 | 1 vial,<br>100 mg       |
| 2             | red   | Pefabloc SC <sup>®</sup> PLUS,<br>PSC-Protector solution   | Ready-to-use solution<br>containing ethanol. |                | 1 bottle,<br>5 ml       |
| <b>Set 2</b>  |       |  |  |                |                         |
| 1             | black | Pefabloc SC <sup>®</sup> PLUS,<br>Pefabloc SC <sup>®</sup> | White powder                                 | 11 873 628 001 | 1 vial,<br>1 g          |
| 2             | black | Pefabloc SC <sup>®</sup> PLUS,<br>PSC-Protector solution   | Ready-to-use solution<br>containing ethanol. |                | 2 bottles,<br>5 ml each |

## 1.2. Storage and Stability

### Storage Conditions (Product)

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

| Vial / Bottle | Label  | Storage   |
|---------------|--|---|
| 1             | Pefabloc <sup>®</sup> SC,<br>Sets I and Set II | Store at +2 to +8°C.<br><b>⚠ Keep protected from light.</b>   |
| 2             | PSC-Protector solution,<br>Sets I and Set II   | Store at +2 to +8°C.<br><b>⚠ Keep protected from light.</b><br><b>⚠ Precipitation may occur during storage. Dissolve precipitates by warming to +15 to +25°C.</b> |

### Storage Conditions (Working Solution)

Store aqueous solutions at neutral pH for 1 to 2 months at –15 to –25°C. Slight hydrolysis occurs under weak, basic conditions (pH 8 to 9).

### Reconstitution

Pefabloc<sup>®</sup> SC is soluble in water or aqueous buffer (0.1 M).

## 1.3. Application

Pefabloc<sup>®</sup> SC is a potent, irreversible serine protease inhibitor and can be used as a non-toxic substitution of phenylmethylsulfonyl fluoride (PMSF) and diisopropylfluorophosphate (DFP).

## 2. How to Use this Product

### 2.1. Before you Begin


#### General Considerations

##### Classes of proteases

Proteases can be assigned to various classes on the basis of their characteristic active centers:

| Protease Type | Active Center  |
|---------------|--|
| Serine        | Serine and histidine   |
| Cysteine      | Cysteine (thiol, SH-)  |
| Metallo       | Metal ions, such as Zn <sup>2+</sup> , Ca <sup>2+</sup> , Mn <sup>2+</sup> |
| Aspartate     | Aspartic acid moiety   |

##### Protease classes and their specific inhibitors

| Serine   | Cysteine | Metallo                    | Aspartate  |
|--|----------|----------------------------|------------|
| Aprotinin*   | E-64*    | Bestatin (aminopeptidase)* | Pepstatin* |
| Pefabloc® SC*  |          | Phosphoramidon             |            |
| Pefabloc® SC PLUS*   |          |                            |            |
| Leupeptin*   |          |                            |            |
|  <i>Inhibits serine and cysteine proteases with trypsin-like specificity.</i> |          |                            |            |
| PMSE*  |          |                            |            |
| cOmplete Protease Inhibitor Cocktail Tablets, EDTA-free*   |          |                            |            |
| cOmplete Protease Inhibitor Cocktail tablets*  |          |                            |            |
| α2-Macroglobulin* (endoproteinases)  |          |                            |            |

#### Removal of the Protector components

The components of the PSC-Protector solution are of low molecular weight and can therefore be easily removed by one of the following methods:

- Dialysis
- Gel chromatography
- Nanofiltration

## Safety Information

### 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](http://dialog.roche.com), or upon request from the local Roche office.

## Working Solution

### Protector solution

The amount of Protector solution depends on the chosen concentration of Pefabloc® SC. Examples for the calculation of the required quantity are shown in the following table:

| Molarity Pefabloc® SC [mM] | Total Volume of extraction solution [ml] | Amount of Pefabloc® SC [mg] | Amount of PSC-Protector solution [ml] |
|----------------------------|--|-----------------------------|---------------------------------------|
| 0.4                        | 100                                      | 10                          | 0.5                                   |
| 4.0                        | 100                                      | 100                         | 5                                     |

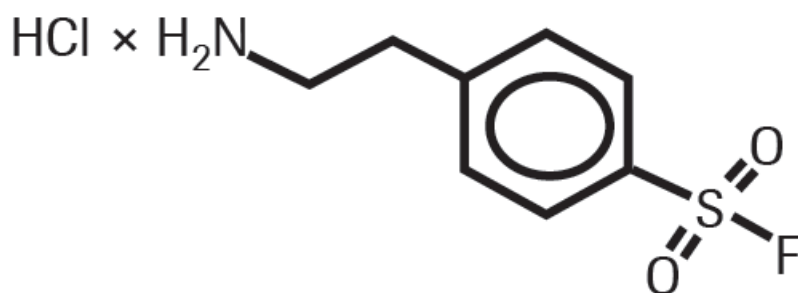
## 2.2. Parameters

### Chemical Formula



### Chemical Name

### Structural formula



**Fig. 1:** Chemical structure of Pefabloc® SC.

### Molecular Weight

239.7 Da

The protector has a molecular weight of less than 150 g/mol.

### Toxicity

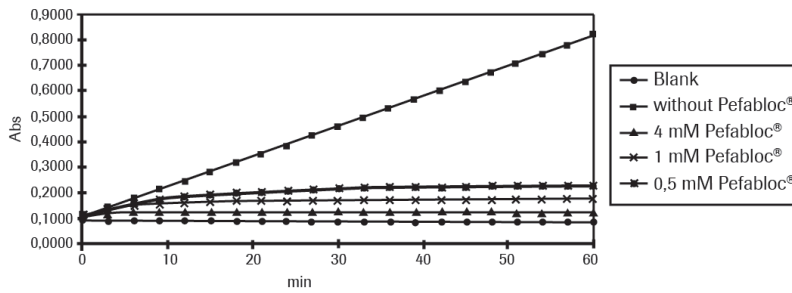
LD<sub>50</sub> = 2.8 g/kg (oral)

### Working Concentration

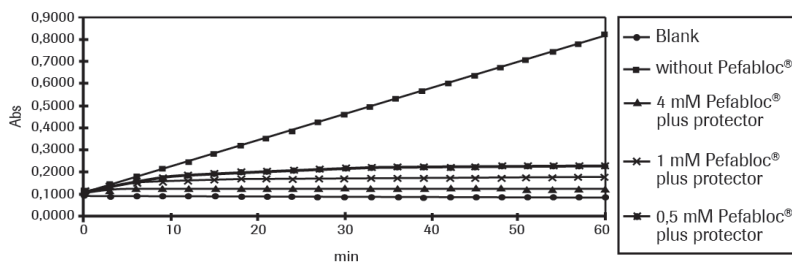
0.1 to 1.0 mg/ml (0.4 to 4 mM)

### 3. Results

The PSC-Protector does not alter the inhibitory activity of Pefabloc® SC as shown in Figures 2 and 3. The inhibition kinetics of Pefabloc® SC alone or in combination with PSC-Protector solution are almost identical.



**Fig. 2:** Chymotrypsin inhibition with Pefabloc\* SC.  
Substrate: Suc-Ala-Ala-Pro-Phe-4-Nitranilide



**Fig. 3:** Chymotrypsin inhibition with Pefabloc\* SC in the presence of PSC-Protector.  
Substrate: Suc-Ala-Ala-Pro-Phe-4-Nitranilide

## 4. Additional Information on this Product

### 4.1. Test Principle

For many years, mechanism-based inhibitors of the class of sulfonyl fluorides, such as PMSF, Pefabloc® SC, or APMSF (4-amidinophenyl-methanesulfonyl fluoride) are successfully used for the inhibition of serine proteases. They react covalently with the serine residue at the catalytic center because its pKa is decreased by the triade geometry increasing dramatically its reactivity.

- Pefabloc® SC is the most potent inhibitor. In contrast to PMSF and APMSF, it shows negligible toxicity and is only very slowly hydrolyzed under weak basic conditions (pH 8 to 9). Also, after the covalent reaction with the serine residue at the catalytic site, negligible hydrolysis back to the active protease is observed. Therefore, Pefabloc® SC is not only suitable for small volumes, such as for the inhibition of isolated serine proteases but also for large volumes, such as the work-ups of fermentations, extractions of animal tissues, plants etc.
- Further advantages are the good solubility in water or aqueous media (PMSF must be dissolved in organic solvents such as DMF, DMSO or ethanol), and its selectivity, for example, the inhibitory activity related to thrombin activity is not delayed in the presence of serum albumin. Therefore, Pefabloc® SC is well suited for the application in natural samples such as serum or plasma.
- Sometimes, nonspecific reactions of these sulfonyl fluoride type inhibitors giving covalent adducts with proteins are observed. This can be analyzed by sequencing and mass spectroscopy. Tyrosine and lysine residues as well as the free amino terminus (N-terminus) can be affected. These side reactions are mainly influenced by the environment of the corresponding amino acid in the protein, the pH of the medium, and the inhibitor/protein ratio (concentration). These side reactions can be suppressed by the PSC-Protector in the set without affecting the specificity and effectiveness of Pefabloc® SC, see section, **Results, Figures 2 and 3**. The composition of the Protector is hereby optimized for the use of Pefabloc® SC. In general, the Protector should be applied to protein solutions with a pH of 7 and higher. If the protein concentration is below 100 µg/ml, the Protector should be used at pH values >6.5.

### Inhibitory activity of Pefabloc® SC



Inhibitory activity of Pefabloc® SC in comparison to PMSF\* on several serine proteases is shown in the following table. Compared to PMSF, it shows a broader inhibitory activity.

| Serine Protease    | $k_{app} / I$ ( $l \times mol^{-1} \times s^{-1}$ ) |       |
|--------------------|---|-------|
|                    | Pefabloc® SC  | PMSF  |
| Trypsin            | 14  | 2.57  |
| Chymotrypsin       | 18.7  | 25.0  |
| Plasmin            | 0.36  | <0.05 |
| Plasmin kallikrein | 0.68  | 0.07  |
| Thrombin           | 1.62  | 1.95  |

## 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>                      |
|  | <b>Important Note: Information critical to the success of the current procedure or use of the product.</b> |
| 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.       |
|---------------------------|---|----------------|
| Non-finished products     |   |                |
| cOplete, EDTA free        | 7500 tablets in glass vial                    | 04 574 834 001 |
| Reagents, kits            |   |                |
| E-64, Protease Inhibitor  | 10 mg   | 10 874 523 001 |
|                           | 25 mg   | 11 585 681 001 |
| Pefabloc SC (AEBSF)       | custom fill                                   | 11 427 393 103 |
| 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 |
|                           | 20 tablets, for 50 ml each                    | 04 693 116 001 |
| cOplete, Mini             | 25 tablets in a glass vial, for 10 ml each    | 11 836 153 001 |
|                           | 30 tablets, for 10 ml each                    | 04 693 124 001 |
| cOplete, Mini, EDTA-free  | 25 tablets in a glass vial, for 10 ml each    | 11 836 170 001 |
| Leupeptin                 | Minimum order quantity 200 mg,<br>custom fill | 10 528 595 103 |
| cOplete, Mini, EDTA-free  | 30 tablets, for 10 ml each                    | 04 693 159 001 |
| Pepstatin                 | custom fill                                   | 10 253 294 103 |
| cOplete, EDTA-free        | 20 tablets, for 50 ml each                    | 04 693 132 001 |
| Aprotinin                 | custom fill                                   | 10 236 632 103 |
| Bestatin                  | 10 mg   | 10 874 515 001 |
| $\alpha_2$ -Macroglobulin | 25 inhibitor units                            | 10 602 442 001 |
| PMSF                      | 10 g  | 10 837 091 001 |
|                           | 25 g  | 11 359 061 001 |



## 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.

