

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



Pefabloc[®] SC

AEBSF, 4-(2-Aminoethyl)-benzenesulfonyl fluoride hydrochloride

 **Version: 17**

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Cat. No. 11 429 868 001	100 mg
Cat. No. 11 585 916 001	500 mg
Cat. No. 11 429 876 001	1 g

Store the product at +2 to +8°C.

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1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Pefabloc® SC (AEBSF)	White crystalline powder	11 429 876 001	1 vial, 1 g
			11 429 868 001	1 vial, 100 mg
			11 585 916 001	1 vial, 500 mg

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® SC (AEBSF)	Store at +2 to +8°C. ⚠ Store dry.

Storage Conditions (Working Solution)

Stability of Pefabloc® SC is affected by both pH and temperature.

- Concentrated stock solutions (100 mM) prepared in double-distilled water are acidic; store for at least two months at –15 to –25°C.
- Pefabloc® SC undergoes hydrolysis at ≥pH 7 and the rate is increased at higher temperatures.
- At pH 7 and +37°C, Pefabloc® SC is hydrolyzed by 50% in 6 hours. However, at the same temperature and at a pH of 7.5, the half life is only 2 hours.]

⚠ Store Pefabloc® SC under acidic conditions and add to biological samples shortly before use to minimize hydrolysis.

Reconstitution

Pefabloc® SC is soluble in aqueous buffers or water (100 mg/ml).

1.3. Application

Pefabloc® SC is a potent serine/threonine phosphatase inhibitor and can be used to:

- Inhibit the detrimental effects of proteases during preparative protein purification.
- Substitute for phenylmethylsulfonyl fluoride (PMSF) and diisopropylfluorophosphate (DFP) due to its markedly lower toxicity, better stability at physiological pH values, and capacity to inhibit a broad range of serine proteases.
- Use in the production of recombinant proteins and during fermentation of transformed cells where proteolytic digestion may decrease the yield of the desired product, all due to its relatively low toxicity towards eukaryotic cells.
- Completely inactivate proteinase K during the preparation of chromosomal DNA in agarose plugs. In this case, the cells are embedded in agarose and digested with proteinase K to degrade all proteins. Before a specific restriction endonuclease is added, the proteinase K is inactivated by incubating the agarose plugs in 1 to 5 mM Pefabloc® SC in 10 mM Tris-HCl, 1 mM EDTA, pH 7 for 2 hours at +37°C or overnight.
- Block thrombin activity in serum or plasma. In these biological fluids, PMSF interacts in a reversible manner with albumin which reduces its free concentration and leads to a delay in thrombin inactivation. Pefabloc® SC however, does not react with serum albumin and exhibits therefore a three-fold higher capacity to inactivate thrombin under similar conditions.

i Pefabloc® SC can be used on living cells.

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 ²⁺ , Ca ²⁺ , Mn ²⁺
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>			
PMSF*			
cOmplete Protease Inhibitor Cocktail Tablets, EDTA-free*			
cOmplete Protease Inhibitor Cocktail tablets*			
α2-Macroglobulin* (endoproteinases)			

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, or upon request from the local Roche office.

2.2. Parameters

Chemical Formula

$C_8H_{10}NO_2SF \times HCl$

Chemical Name

Structural formula

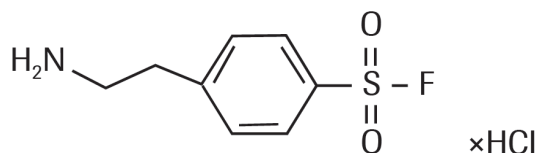


Fig. 1: Chemical structure of Pefabloc® SC.

Molecular Weight

239.5 Da

Specificity

Pefabloc® SC belongs to the family of sulfonyl fluorides which irreversibly block serine proteases. See section, **General Considerations** for a table of protease classes and their inhibitors.

Toxicity

$LD_{50} = 2.8 \text{ g/kg}$ (oral dose in mice)

Working Concentration

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

⚠ Add to biological samples directly before use to minimize hydrolysis.

i Protease inhibitors of the sulfonyl fluoride type such as PMSE, APMSF, and Pefabloc® SC can, depending on the protein structure and concentration, form covalent adducts with proteins if incubated at certain pH values in higher concentrations. Use Pefabloc® SC PLUS* to circumvent these adduct formations without altering the inhibitory features. The protector solution is available together with the inhibitor as a set.

3. Additional Information on this Product

3.1. Test Principle

Mechanism of action

Serine proteases, one of several subclasses of proteases, are characterized by a serine residue, present at the active center of the protease.

- This serine residue is reactive and participates in the catalytic mechanism during peptide bond hydrolysis.
- The fluoro-phosphate inhibitors such as DFP and sulfonyl fluorides, such as Pefabloc® SC or PMSF*, rapidly react with the serine residue, forming a stable acylated enzyme resulting in a loss of proteolytic activity.
- The inhibitors vary in many respects, including specificity for particular proteases and the rate at which they inactivate these proteases.
- Pefabloc® SC inhibits a broad range of serine proteases with an efficiency which is better or equivalent to other commonly used inhibitors, see **Inhibitory activity of Pefabloc® SC**.

Inhibitory activity of Pefabloc® SC

Inhibitory activity of Pefabloc® SC in comparison to PMSF* and DFP on several serine proteases is shown in the following table.

k_{app}/I is the apparent rate constant of inactivation of the protease as a function of inhibitor concentration. Pefabloc® SC inactivates plasmin, plasma kallikrein, thrombin, and glandular kallikrein faster than either PMSF or DFP. Trypsin and chymotrypsin are inactivated at a comparable rate.

Serine Protease	k_{app}/I ($l \times mol^{-1} \times s^{-1}$)		
	Pefabloc® SC	PMSF	DFP
Trypsin	3.06	2.57	6.23
Chymotrypsin	17.8	25.0	39.0
Plasmin	0.32	0.05	0.19
Plasmin kallikrein	0.68	0.07	0.3
Thrombin	5.12	1.95	1.28
Glandular kallikrein	0.19	0.05	0.05
TPA	1.19	Not detected	Not detected
Subtilisin A	0.46	Not detected	Not detected

3.2. Quality Control

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

4. Supplementary Information

4.1. 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.*

⚠ 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.

4.2. Changes to previous version

Layout changes.

Editorial changes.

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

4.3. Ordering Information

Product	Pack Size	Cat. No.
Non-finished products		
cComplete, 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
cComplete	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
cComplete, 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
cComplete, Mini, EDTA-free	25 tablets in a glass vial, for 10 ml each	11 836 170 001
Leupeptin	Minimum order quantity 200 mg, custom fill	10 528 595 103
cComplete, Mini, EDTA-free	30 tablets, for 10 ml each	04 693 159 001
Pepstatin	custom fill	10 253 294 103
cComplete, 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
Pefabloc® SC PLUS	Set I, 100 mg Pefabloc® SC; 5 ml PSC-Protector solution	11 873 601 001
	Set II, 1 g Pefabloc® SC; 2 x 25 ml PSC-Protector solution	11 873 628 001
α ₂ -Macroglobulin	25 inhibitor units	10 602 442 001
PMSF	10 g	10 837 091 001
	25 g	11 359 061 001

4. Supplementary Information

4.4. Trademarks

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

4.5. License Disclaimer

For patent license limitations for individual products please refer to:

List of biochemical reagent products.

4.6. Regulatory Disclaimer

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

4.7. Safety Data Sheet

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

4.8. 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.

