

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



E-64, Protease Inhibitor

N-[N-(L-3-Trans-carboxirane-2-carbonyl)-L-leucyl]-agmatine

 **Version: 08**

Content Version: October 2019

Crystallized

Cat. No. 10 874 523 001 10 mg

Cat. No. 11 585 681 001 25 mg

Store the product at +2 to +8°C.

1.	General Information	3
1.1.	Contents	3
1.2.	Storage and Stability	3
	Storage Conditions (Product)	3
	Storage Conditions (Working Solution).....	3
	Reconstitution	3
1.3.	Application	3
2.	How to Use this Product	4
2.1.	Before you Begin	4
	General Considerations.....	4
	Protease classes and their specific inhibitors.....	4
2.2.	Parameters	5
	Chemical Formula.....	5
	Chemical Name.....	5
	Structural formula.....	5
	Inhibition	5
	Mechanism of inhibition	5
	Molecular Weight	5
	pH Stability.....	5
	Purity.....	5
	Specificity	5
	Toxicity	5
	Working Concentration.....	5
3.	Additional Information on this Product	6
3.1.	Test Principle	6
	Preparation.....	6
4.	Supplementary Information	7
4.1.	Conventions.....	7
4.2.	Changes to previous version.....	7
4.3.	Ordering Information.....	7
4.4.	Trademarks.....	8
4.5.	License Disclaimer	8
4.6.	Regulatory Disclaimer.....	8
4.7.	Safety Data Sheet.....	8
4.8.	Contact and Support.....	8

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	E-64, Protease Inhibitor	Thiol protease inhibitor	10 874 523 001	1 vial, 10 mg
			11 585 681 001	1 vial, 25 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	E-64, Protease Inhibitor	Store at +2 to +8°C.

Storage Conditions (Working Solution)

Store E-64 in neutral water/methanol or ethanol solutions (1:1) for one day at +2 to +8°C or for 1 to 2 months at –15 to –25°C.

⚠ E-64 may be degraded by hydrolysis in solution at +15 to +25°C.

Reconstitution

E-64 is soluble to 20 mg/ml in a 1:1 mixture of ethanol and water.

i *Vortexing or slight warming in a water bath at +40°C may facilitate dissolution.*

- It is also soluble in a neutral water/methanol solution, in water, methanol, acetic acid, pyridine, and DMSO.
- Sparingly soluble in ethanol and propanol.
- Insoluble in acetone, chloroform, ethyl ether, and benzene.

1.3. Application

E-64 is used for the isolation and purification of proteins and enzymes.

2. How to Use this Product

2.1. Before you Begin

General Considerations

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

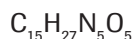
Protease Type	Active Center
Serine	Serine and histidine
Cysteine	Cysteine (thiol, SH-)
Metallo	Metal ions, such as Zn ²⁺ , Ca ²⁺ , Mn ²⁺
Aspartate	Aspartic acid moiety

Protease classes and their specific inhibitors

Serine	Cysteine	Metallo	Aspartate
Aprotinin*	E-64*	Bestatin (aminopeptidase)*	Pepstatin*
Pefabloc® SC*		Phosphoramidon	
Pefabloc® SC PLUS*			
Leupeptin*			
<i>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)			

2.2. Parameters

Chemical Formula



Chemical Name

Structural formula

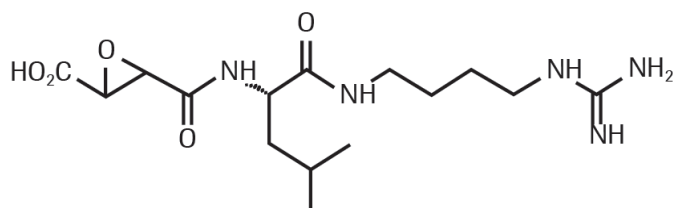


Fig. 1: Chemical structure of E-64.

Inhibition

Mechanism of inhibition

The carbonyl group and the epoxide group appear to be responsible for the inhibitory effect of E-64.

Molecular Weight

357.4 Da

pH Stability

The working solution is stable between pH 2 and pH 10, but not in extremely acidic or alkaline buffers.

Purity

>95% (HPLC)

Specificity

The thiol protease inhibitor E-64 specifically inhibits papain and other cysteine proteases such as cathepsin B and L, bromelain, and ficin.

- Cathepsin A and D are not inhibited.
- L-lactate dehydrogenase from porcine heart is not inhibited although the enzyme contains a functional thiol group.
- Inhibition of thiol proteases by E-64 appears to be of a non-competitive nature between the SH components.
- The inhibition is also irreversible, and is altered after gel filtration (Sephadex column) or dialysis after incubation of papain with E-64. The enzyme and inhibitor combine in an equimolar ratio.

See section, **General Considerations** for a table of protease classes and their inhibitors.

Toxicity

LD₅₀: >2,000 mg/kg (mouse and rat)

Working Concentration

0.5 to 10 µg/ml (1.4 to 28.0 µM)

3. Additional Information on this Product

3.1. Test Principle

Preparation

E-64 was first isolated from culture extracts of *Aspergillus japonicus* TPR-64. It is obtained as crystals in the form of white needles and is electrophoretically neutral. Pronase E digestion cleaves E-64 into three components which can be isolated in a crystalline form. The three components are L-leucine, agmatin, and L-trans-succinic acid.

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

 *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.

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		
Pefabloc® SC (AEBSF)	custom fill	11 427 393 103
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	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

COMPLETE is a trademark of Roche.

All other 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.

