Endoproteinase Arg-C

Sequencing Grade
From *Clostridium histolyticum*

Cat. No. 11 370 529 001 3 × 5 µg

Version 17
Content version: May 2013
Store at +2 to +8°C

1. What this Product Does

Content

Lyophilizate

One pack contains:

- 3 vials with Endoproteinase Arg-C (Vial 1)
- 3 vials of lyophilized activation solution (Vial 2)

Storage and Stability

The lyophilizate is stable at +2 to +8°C until the expiration date printed on the label. The working solution of Endoproteinase Arg-C in redistilled water may be used for a maximum of 5 days, when stored at +2 to +8°C.

The reconstituted activation solution is stable for approximately 1 day, when stored at +2 to +8°C.

For long term use, the solution should be gently flushed with a stream of nitrogen and stored at —15 to —25°C.

Store dry!

Application

Use Endoproteinase Arg-C for the specific cleavage of proteins and peptides for peptide mapping, fingerprinting, and sequence analysis. The protease is suitable for digesting proteins in solution, in gels, or on blotting membranes.

2. How to Use this Product

2.1 Before You Begin

General Handling Recommendations

The content of one vial may be used for several simultaneous digests. A new vial should be taken when repeating a digest in order to minimize the risk of contamination or autolysis.

2.2 Digestion of Proteins in Solution

Working Solution

Reconstitute lyophilized Endoproteinase Arg-C in 50 µl redistilled water to give a final concentration of 50 mM Tris/HCl buffer, 10 mM $CaCl_2$, 5 mM EDTA, pH 8.0. Dissolve the lyophilized activation solution in 100 µl redistilled water, resulting in a final 50 mM Dithiothreitol-(DTT) and 5 mM EDTA concentration.

Procedure

Step	Action	
❶	Dissolve the proteins to be sequenced in digestion buffer (100 mM Tris/HCl, 10 mM $CaCl_2$, pH 7.6).	
❷	In the case of proteins that are hard to solubilize, add urea, SDS or guanidine hydrochloride to the digestion buffer prior to solubilizing the protein. When applying urea, Roche recommends also adding 20 mM methylamine.	
❸	To achieve a suitable concentration of the denaturing agent in the digest, the protein solution should be correspondingly diluted with buffer (Tab. 1).	
❹	For a typical digest, mix the solutions in the following way:	
	Endoproteinase Arg-C	5 µl
	Proteins diluted in digestion buffer	5 - 85 µl
	Activation solution	10 µl
	Digestion buffer	add 100 µl
	Under these conditions the digestion mixture contains the following concentration: 90 mM Tris/HCl buffer, 8,5 mM $CaCl_2$, 5 mM DTT, 0.5 mM EDTA, pH 7.6.	
❺	The recommended amount of enzyme is 1/200 to 1/50 of the protein by weight.	
❻	The incubation time should be chosen between 1 and 18 h at +37°C depending on the amount of enzyme.	

Tab. 1: Activity determination of Endoproteinase Arg-C, with BAEE (N-a-Benzoyl-L-Arginine ethylester) as substrate in the presence of stated concentrations of denaturing agents. Incubation of Endoproteinase Arg-C, with denaturing agent for 4 h at ambient temperature in 50 mM Tris/HCl buffer, 1 mM DTT.

Roche recommends also adding 20 mM methylamine when applying urea.

Denaturing agent	Concentration	Enzyme activity in %
without addition (control)	-	100
sodium dodecyl sulfate (SDS) [%(w/v)]	0.5	3
	0.1	4
	0.01	57
urea [mol/l] (+ methylamine)	4	140
	1	130
	0.5	130
	0.1	125
guanidine hydrochloride [M]	4	-
	1	4
	0.5	16
	0.1	26
acetonitrile [%(v/v)]	10	115
	5	126
	1	130

3. Additional Information on this Product

3.1 Product Characteristics

Source

Endoproteinase Arg-C is isolated from *Clostridium histolyticum* as a highly purified and specific protease.

Sequence of Endoproteinase Arg-C (3,4)

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        10         20         30         40         50         60
MLRRKVSTLL MTALITTSFL NSKPVYANPV TKSKDNNLKE VQQVTSKSNK NKNQKVTIMY

        70         80         90        100        110        120
YCDADNNLEG SLLNDIEEMK TGYKDSPNLN LIALVDRSPR YSSDEKVLGE DFSDTRLYKI

       130        140        150        160        170        180
EHNKANRLDG KNEFPEISTT SKYEANMGDP EVLKKFIDYC KSNYEADKYV LIMANHGGGA

       190        200        210        220        230        240
REKSNPRLNR AICWDDSNLD KNGEADCLYM GEISDHLTEK QSVDLLAFDA CLMGTAEVAY

       250        260        270        280        290        300
QYRPGNGGFS ADTLVASSPV VWGPGFKYDK IFDRIKAGGG TNNEDDLTLG GKEQNFDPAT

       310        320        330        340        350        360
ITNEQLGALF VEEQRDSTHA NGRYDQHLSF YDLKKAESVK RAIDNLAVNL SNENKKSEIE

       370        380        390        400        410        420
KLRGSGIHTD LMHYFDEYSE GEWVEYPYFD VYDLCEKINK SENFSSKTKD LASNAMNKLN

       430        440        450        460        470        480
EMIVYSFGDP SNNFKEGKNG LSIFLPNGDK KYSTYYTSTK IPHWTMQSWY NSIDTVKYGL

       490        500        510        520
NPYGKLSWCK DGQDPEINKV GNWFELLDSW FDKTNDVTGG VNHYQW
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3.2 Quality control

Function and purity control by HPLC of each lot ensure a constant quality.

According to the current quality control procedures, the enzyme is free of impurities that might interfere with the separation range of peptides in reversed-phase HPLC (detection at 215 nm).

Specificity and Nonspecificity Verification

Endoproteinase Arg-C is a cysteine serine protease that specifically hydrolyzes proteins and peptide bonds C-terminally of arginine residues.

The specificity is confined primarily to arginine residues, although hydrolysis proceeds to a minor degree in most lysine-containing substrates (1,2).

The specificity and nonspecificity of Endoproteinase Arg-C is verified with the oxidized B-chain of insulin (insulin B_{ox}) as substrate.

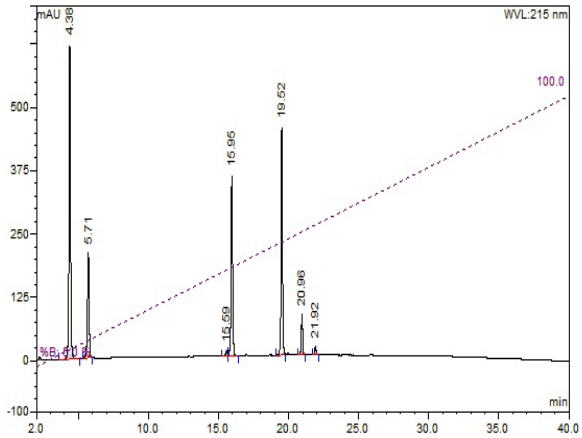

Fig. 1: Specificity of Endoproteinase Arg-C in reversed phase HPLC. High concentrations of Endoproteinase Arg-C (1 part by weight enzyme with 200 parts by weight insulin B_{ox}) are incubated for 1 h to detect the fragments of the specific digested substrate.

Digest	20 µg insulin B_{ox} in 80 µl 84.4 mM Tris/HCl buffer with 8.44 mM CaCl2, 4.96 mM DTT, 0.469 mM EDTA at pH 7.6 + 0.1 µg (20 µl) Endoproteinase Arg-C dissolved in water to 0.1 µg/µl, diluted 1:20 with 100 mM Tris/HCl buffer, 10 mM $CaCl_2$; 1 h at +37°C; reversed phase HPLC: undiluted.
Column	Nucleosil 100-5-C18 4 x 100 mm, 5 µm
Solvent A	0.1% TFA (v/v) in water
Solvent B	0.1% TFA (v/v) in water; 70% acetonitrile (v/v)
Gradient	40 min linearly 0-100% B;
Flow rate	1 ml/min
Wavelength:	215 nm
Fragments	15.95 min Gly (23) –Ala (30) 19.52 min Phe (1) – Arg (22)

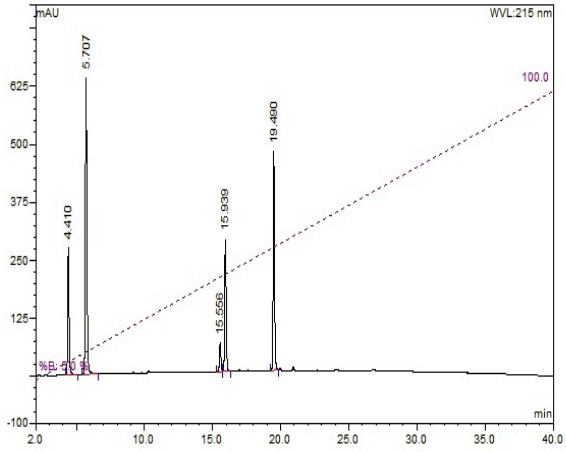

Fig. 2: Nonspecificity of Endoproteinase Arg-C in reversed phase HPLC. High concentrations of Endoproteinase Arg-C (1 part by weight enzyme with 10 parts by weight insulin B_{ox}) are incubated for 18 h to detect traces of impurities.

Digest	20 µg insulin B_{ox} in 80 µl 84.4 mM Tris/HCl buffer with 8.44 mM $CaCl_2$, 4.96 mM DTT, 0.469 mM EDTA at pH 7.6 + 2.0 µg Endoproteinase Arg-C dissolved in 20 µl water; 18 h at +37°C; reversed phase HPLC: undiluted.
Column	Nucleosil 100-5-C18 4 x 100 mm, 5 µm
Solvent A	0.1% TFA (v/v) in water
Solvent B	0.1% TFA (v/v) in water; 70% acetonitrile (v/v)
Gradient	40 min linearly 0-100% B;
Flow rate	1 ml/min
Wavelength:	215 nm
Fragments	15.94 min Gly (23) –Ala (30) 19.49 min Phe (1) – Arg (22)

References

1 Mitchell, W. M. & Harrington, W. F. (1971) *The Enzymes III* (Boyer, P.D., ed.) 699-719

2 Mitchell, W. M. & Harrington, W. F. (1968) *J. Biol. Chem.* **243**, 4683-4692

3 Dargatz, H. *et al.* (1993) *Mol. Gen. Genet.* **240**, 140-145

4 Swiss-Prot: P09870; (Jun. 2010)

4. Supplementary Information

Changes to Previous Version

- Update of Quality control data
- Editorial changes

Text Conventions

To make information consistent and understandable, the following text conventions are used in this document:

Text Convention	Use
Numbered instructions labeled ❶, ❷, etc.	Stages in a process that usually occur in the order listed.

Symbols

Symbols are used in this document to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.

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