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

Endoproteinase Arg-C Sequencing Grade
from *Clostridium histolyticum*

Version: 19
Content Version: November 2020

Lyophilized

Cat. No. 11 370 529 001 3 x 5 µg

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	Endoproteinase Arg-C Sequencing Grade	Highly purified and specific protease.	3 vials, 5 µg each
2	Endoproteinase Arg-C Sequencing Grade, Activation solution	For digestion mixture.	3 vials

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	Endoproteinase Arg-C Sequencing Grade	Store at +2 to +8°C. ⚠ ***Store dry.***
2	Activation solution	

1.3. Additional Equipment and Reagent required

For preparation of digestion buffer

See section, **Working Solution** *for additional information on preparing solutions*

- Tris-HCl*
- $CaCl_2$

For solubilization of proteins

- Sodium dodecyl sulfate (SDS*)
- Urea
- Methylamine
- Guanidine hydrochloride
- Acetonitrile

1.4. Application

Use Endoproteinase Arg-C for the specific cleavage of proteins and peptides for:

- Peptide mapping
- Fingerprinting
- Sequence analysis

The protease is suitable for digesting proteins in solution, gels, or on blotting membranes.

2. How to Use this Product

2.1. Before you Begin

General Considerations

General handling

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

Activity determination

Activity determination of Endoproteinase Arg-C, with BAEE (N-α-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 hours at ambient temperature in 50 mM Tris-HCl buffer, 1 mM DTT.

Add 20 mM methylamine when applying urea.

Denaturing agent	Concentration	Enzyme activity [%]
Without addition (control)	–	100
SDS	0.5% (w/v)	3
	0.1% (w/v)	4
	0.01% (w/v)	57
Urea (+ methylamine)	4 M	140
	1 M	130
	0.5 M	130
	0.1 M	125
Guanidine hydrochloride	4 M	–
	1 M	4
	0.5 M	16
	0.1 M	26
Acetonitrile	10% (v/v)	115
	5% (v/v)	126
	1% (v/v)	130

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.

Working Solution

Solution	Preparation/Composition	Storage and Stability	For use in...
Endoproteinase Arg-C Sequencing Grade	▪ Add 50 µl double-distilled water to the lyophilizate. ▪ Results in a final concentration of 50 mM Tris-HCl buffer, 10 mM $CaCl_2$, 5 mM EDTA, pH 8.0.	Store 5 days at +2 to +8°C.	Digestion mixture
Activation solution	▪ Add 100 µl double-distilled water to the lyophilizate. ▪ Results in a final concentration of 50 mM Dithiothreitol (DTT) and 5 mM EDTA.	Store 1 day at +2 to +8°C. For long-term storage, gently flush the solution with a stream of nitrogen and store at −15 to −25°C.	Digestion mixture
Digestion buffer	100 mM Tris-HCl*, 10 mM $CaCl_2$, pH 7.6.	–	Dissolution of the proteins to be sequenced.

2.2. Protocols

Digestion of proteins in solution

See section, **Working Solution** *for information on preparing solutions.*

1. Dissolve the proteins to be sequenced in Digestion buffer.
   For proteins that are hard to solubilize, add urea, SDS, or guanidine hydrochloride to the Digestion buffer prior to solubilizing the protein. When applying urea, also add 20 mM methylamine.

2. Dilute protein solution with buffer, see section, **General Considerations** to achieve a suitable concentration of the denaturing agent in the digest.

3. For a typical digest, mix the solutions as shown in the table:

Solution	Volume [µl]
Endoproteinase Arg-C	5
Proteins diluted in Digestion buffer	5 – 85
Activation solution	10
Digestion buffer	100

   Under these conditions, the digestion mixture contains a concentration of 90 mM Tris-HCl buffer, 8.5 mM $CaCl_2$, 5 mM DTT, 0.5 mM EDTA, pH 7.6.

4. The recommended amount of enzyme is 1/200 to 1/50 of the protein by weight.

5. Choose an incubation time between 1 and 18 hours at +37°C, depending on the amount of enzyme.

2.3. Parameters

Sequence

Sequence of Endoproteinase Arg-C

1					
MLRRKVSTLL	MTALITTSFL	NSKPVYANPV	TKSKDNNLKE	VQQVTSKSNK	NKNQKVTIMY
61					
YCDADNNLEG	SLLNDIEEMK	TGYKDSPNLN	LIALVDRSPR	YSSDEKVLGE	DFSDTRLYKI
121					
EHNKANRLDG	KNEFPEISTT	SKYEANMGDP	EVLKKFIDYC	KSNYEADKYV	LIMANHGGGA
181					
REKSNPRLNR	AICWDDSNLD	KNGEADCLYM	GEISDHLTEK	QSVDLLAFDA	CLMGTAEVAY
241					
QYRPGNGGFS	ADTLVASSPV	VWGPGFKYDK	IFDRIKAGGG	TNNEDDLTLG	GKEQNFDPAT
301					
ITNEQLGALF	VEEQRDSTHA	NGRYDQHLSF	YDLKKAESVK	RAIDNLAVNL	SNENKKSEIE
361					
KLRGSGIHTD	LMHYFDEYSE	GEWVEYPYFD	VYDLCEKINK	SENFSSKTKD	LASNAMNKLN
421					
EMIVYSFGDP	SNNFKEGKNG	LSIFLPNGDK	KYSTYYTSTK	IPHWTMQSWY	NSIDTVKYGL
481					
NPYGKLSWCK	DGQDPEINKV	GNWFELLDSW	FDKTNDVTGG	VNHYQW	

3. Additional Information on this Product

3.1. Test Principle

Background information

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

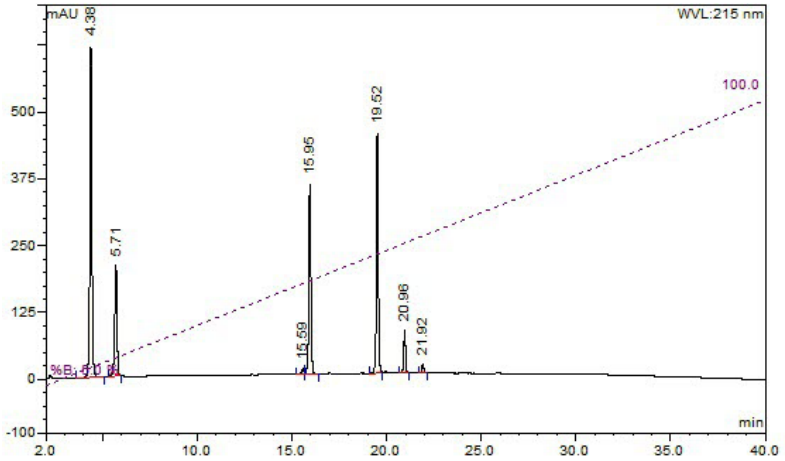

Fig. 1: Specificity of Endoproteinase Arg-C in reversed phase HPLC.
High concentrations of Endoproteinase Arg-C (1 part by weight enzyme with 200 parts by weight insulin B_{ox}) are incubated for 1 hour to detect the fragments of the specific digested substrate.

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

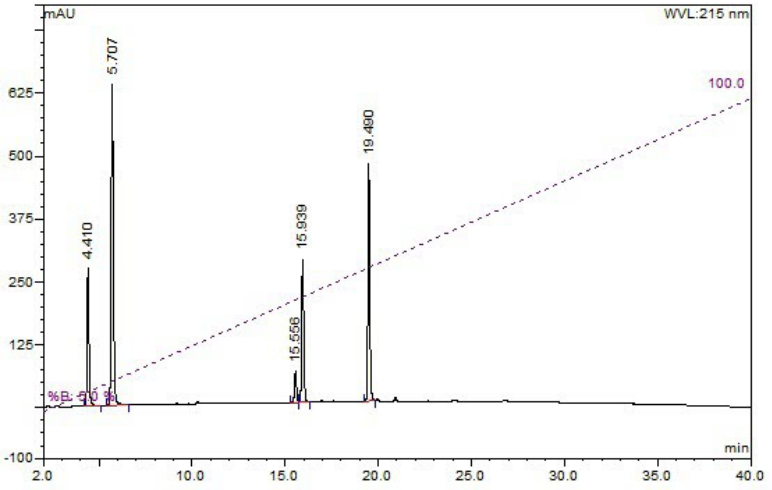

Fig. 2: Nonspecificity of Endoproteinase Arg-C in reversed phase HPLC.
High concentrations of Endoproteinase Arg-C (1 part by weight enzyme with 10 parts by weight insulin B_{ox}) are incubated for 18 hours to detect traces of impurities.

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 double-distilled water; 18 hours at +37°C; reversed phase HPLC: undiluted.
Column	Nucleosil 100-5-C18 4 × 100 mm, 5 µm
Solvent A	0.1% TFA (v/v) in double-distilled water
Solvent B	0.1% TFA (v/v) in double-distilled water; 70% acetonitrile (v/v)
Gradient	40 minutes linearly 0 to 100% B
Flow rate	1 ml/minute
Wavelength	215 nm
Fragments	15.94 min Gly (23) – Ala (30) 19.49 min Phe (1) – Arg (22)

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	
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.
❶ ❷ ❸ 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.
Reagents, kits		
Tris hydrochloride	500 g	10 812 846 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001

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.

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