

Certificate of Analysis

Arg-C, Sequencing Grade:

Part No.	Size
V188A	10µg

Description: Arg-C (clostripain) is an endopeptidase that cleaves at the C-terminus of arginine residues, including sites next to proline. Cleavage also will occur at lysine residues (1–4). This sequencing-grade enzyme can be used alone or in combination with other proteases for protein analysis by mass spectrometry and other applications.

Biological Source: *Clostridium histolyticum*.

Molecular Weight: Composed of two subunits of 45kDa and 12kDa.

Form: Lyophilized.

Storage Conditions: See the Product Information Label for storage conditions and expiration date.

Optimal pH: 7.6–7.9 (1).

Activators: Dithiothreitol, cysteine or another reducing agent and CaCl₂ are required for enzyme activity.

Inhibitors: Oxidizing agents and sulfhydryl reactants; also Co²⁺, Cu²⁺, Cd²⁺ and heavy metal ions. Citrate, borate and Tris anions partially inhibit activity. TLCK (tosyllysine chloromethyl ketone) reacts with active site (1,5,6).

Usage Notes:

1. Resuspend Arg-C in incubation buffer (50mM Tris-HCl [pH 7.6–7.9], 5mM CaCl₂, 2mM EDTA). Store reconstituted Arg-C at –20°C for up to 10 weeks or at 4°C for up to 2 weeks.
2. DTT or another reducing reagent is required for full activity.
3. Arg-C, Sequencing Grade, is lyophilized in the presence of 0.25mg trehalose, 0.1µmol cysteine and 2nmol CaCl₂.

Quality Control Assays

This lot passes the following Quality Control specifications:

Specificity: Following digestion reactions using b-insulin as a substrate at 1:20 and 1:200 protease:substrate ratios performed for 30 minutes at 37°C, reverse-phase HPLC analysis shows complete digestion of the starting material and two Arg-C-specific peaks.

Usage Information on Back

Signed by:

R. Wheeler, Quality Assurance

Part# 9PIV188

Revised 8/16



AF9PIV188 0816V188



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Part# 9PIV188
Printed in USA. Revised 8/16.

1. In-Solution Digestion Protocol

- Resuspend the protein in incubation buffer (see Section 2 for recipe).
Note: Alkylating reagents are inhibitors of Arg-C. If an alkylation reagent was used during sample preparation, quench the unused reagent by adding DTT prior to adding the enzyme.
- Transfer the protein solution to a microcentrifuge tube.
- Resuspend Arg-C protease in incubation buffer.
- Add the enzyme to protein solution; mix. We recommend using enzyme:protein ratios of 1:20 to 1:350.
- Add activation buffer, 10X, to a final concentration of 1X (see Section 2 for recipe).
Note: DTT is an activator for Arg-C and must be added to final concentrations greater than 2mM. DTT or another reducing reagent is required for full activity.
- Centrifuge briefly to collect components at the bottom of the tube.
- Incubate for 2–18 hours at 37°C. Shorter incubation periods are recommended with higher amounts of protease.

Enzyme:Protein Ratio	Digestion Time Guideline
1:20	1–3 hours
1:50	1–4 hours
1:100 or higher	3–18 hours

- Stop the reaction by adding 10% formic acid or TFA (trifluoroacetic acid) to a final concentration of 0.5% or by heating at 95°C for 10 minutes.

2. Composition of Buffers and Solutions

incubation buffer

50mM	Tris-HCl (pH 7.6–7.9)
5mM	CaCl ₂
2mM	EDTA

activation buffer, 10X

50mM	Tris-HCl (pH 7.6–7.9)
50mM	DTT
2mM	EDTA

3. References

- Handbook of Proteolytic Enzymes* (1998) 760.
- Mitchell, W.M. and Harrington, W.F. (1971) In: *The Enzymes III* Boyer, P.D., ed. 699.
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- Meiwees, J. *et al.* (1991) *Biomed. Biochim. Acta* **50**, S80–3.
- Gilles, A.M., Imhoff, J.M. and Keil, B. (1979) *J. Biol. Chem.* **254**, 1462–8.
- Porter, W.H., Cunningham, L.W. and Mitchell, W.M. (1971) *J. Biol. Chem.* **246**, 7675.

4. Related Products

Product	Size	Conc.	Cat. #
Asp-N, Sequencing Grade	2µg		V1621
Chymotrypsin, Sequencing Grade	25µg		V1061
	100µg (4 × 25µg)		V1062
Elastase	5mg		V1891
Endo H	10,000u	500u/µl	V4871
	50,000u	500u/µl	V4875
Endoproteinase Lys-C, Sequencing Grade	5µg		V1071
Fetuin	500µg	10mg/ml	V4961
Glu-C, Sequencing Grade	50µg (5 × 10µg)		V1651
Immobilized Trypsin	2ml		V9012
	4ml (2 × 2ml)		V9013
Pepsin	250mg		V1959
PNGase F	500u	10u/µl	V4831
ProteaseMAX™ Surfactant, Trypsin Enhancer	1mg		V2071
	5mg (5 × 1mg)		V2072
Protein Deglycosylation Mix	20 reactions		V4931
rLys-C, Mass Spec Grade	15µg		V1671
Sequencing Grade Modified Trypsin	100µg (5 × 20µg)		V5111
Sequencing Grade Modified Trypsin, Frozen	100µg (5 × 20µg)		V5113
Thermolysin	25mg		V4001
Trypsin Gold, Mass Spectrometry Grade	100µg		V5280
Trypsin/Lys-C Mix, Mass Spec Grade	20µg		V5071
	100µg		V5072
	100µg (5 × 20µg)		V5073