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ProductInformation

Endoproteinase ARG-C
from mouse submaxillary gland
suitable for Protein Sequencing

Catalog Number **P6056**
Storage Temperature –20 °C

TECHNICAL BULLETIN

CAS RN 82047-85-6
EC: 3.4.21.35

Product Description

Endoproteinase Arg-C is a serine endoprotease from mouse submaxillary gland which hydrolyzes peptide bonds at the carboxyl side of arginyl residues and also exhibits esterase and amidase activities.[1,2] The enzyme has been shown to cleave Lys-Lys and Lys-Arg bonds, and all Arg-X bonds may not be hydrolyzed.[3-6]

Endoproteinase Arg-C is HPLC purified, resulting in a product suitable for proteomic work. In 100 mM NH_4HCO_3, pH 8.5, or 100 mM Tris HCl, pH 8.5, Arg-C specifically cleaves peptide bonds at the carboxyl side of arginine. Arg-C is used in proteomics for peptide mapping and protein sequence work due to its highly specific cleavage of peptides resulting in a limited number of fragments.[1-6]

Self digestion may occur during the alkaline incubation period. The specificity and activity of Arg-C is retained in 20% organic solvents.[7] Endoproteinase Arg-C loses specificity during long incubations. A known peptide such as α-melanocyte stimulating hormone should be run as a control for all experiments.

Endoproteinase Arg-C has an average molecular mass of 26.5 kDa and a pH optimum between pH 7.5 and 8.5.[1,2]

Vial content: 5 µg protein (BCA assay)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Reconstitute the lyophilized product in 1 mM HCl.

Storage/Stability

The lyophilized powder is stable for at least one year if stored unopened in the freezer. After reconstitution in 1 mM HCl, frozen aliquots lose 50% activity after one week.

Procedure

For peptide or protein digestion, a ratio between 1:20 to 1:100 (w/w) of enzyme to substrate is recommended. Dissolve the peptide or protein to be digested in 100 mM NH_4HCO_3, pH 8.5, or 100 mM Tris HCl, pH 8.5. Recommended incubation time is between 2 and 18 hours at 37 °C depending on the enzyme to substrate ratio.

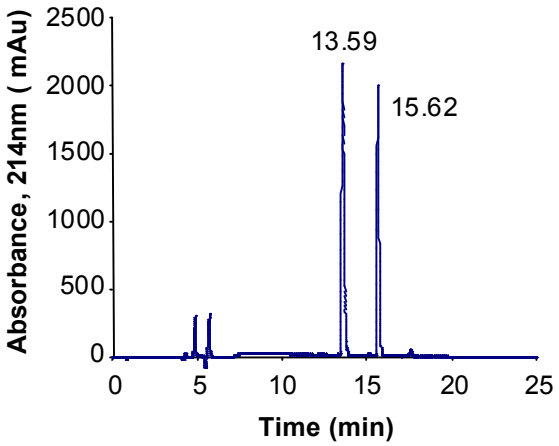
Results

The suitability of this product is demonstrated in Figure 1 by digestion of α-melanocyte stimulating hormone (Catalog Number M4135). The sequence of α-melanocyte stimulating hormone is:

Acetyl-SYSMEHFRWGKPV

During the 18 hour digestion only the expected peptides were generated with no indication of other major proteolytic activity.

Figure 1.
Suitability Assay of ARG-C.



α-melanocyte stimulating hormone (100 µg) was digested with 5 µg of Arg-C for 18 hours at 37 °C in 100 µl of 100 mM NH_4HCO_3, pH 8.5. A 20 µg aliquot was separated on a Supelco Discovery C_{18} column (25 cm x 4.6 mm, 5 µm, Catalog Number 504971) using a 20 minute linear gradient from 5-50% B at 0.7 ml/min with UV detection at 214 nm and by mass spectrometry.
Solvent A: 0.1% (v/v) TFA in water.
Solvent B: 0.08% (v/v) TFA in acetonitrile.

The Arg-C proteolytic fragments were identified as follows:

Retention Time (min)	Mass (Da)	Fragment
13.59	584.3	Trp(9)-Val(13)
15.62	1098.0	Acetyl-Ser(1)-Arg(8)

References

1. Levy, M. *et al*. Meth. Enzymol., **19**, 672-681, (1970).
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3. Chartier, F. *et al*., J. Biol. Chem., **264**, 17006-15, (1989).
4. Bousfield, G.L., and Ward, D.N., J. Biol. Chem., **263**, 12602-7, (1988).
5. Aitken, A. *et al*., Protein Sequencing: A Practical Approach, IRL Press, (Oxford, UK: 1989), p 43.
6. Burdon, R.H., and Knippenberg, P.H., (eds.), Laboratory Techniques in Biochemistry and Molecular Biology: Sequencing of Proteins and Peptides, Volume 9, Elsevier, (New York, NY: 1989) p 73.
7. Welinder, K.G., Anal. Biochem., **174**, 54-64, (1988).

Related Products

SIGMA HPLC Purified Products:

Product	Catalog Number
Carboxypeptidase Y	C4046
α-Chymotrypsin	C6423
Endoproteinase Arg-C	P6056
Endoproteinase Asp-N	P3303
Endoproteinase Glu-C	P6181
Endoproteinase Lys-C	P3428
Leucine aminopeptidase	L9776
Trypsin	T8658
Insulin Chain B, Oxidized	I1764
Melittin	M1407
Glucagon	G7774
α-Melanocyte Stimulating Hormone	M4135

Titles of Related Books (Catalog Number):
1. MICROCHARACTERIZATION OF PROTEINS (M9807)
2. NEW METHODS IN PEPTIDE MAPPING FOR THE CHARACTERIZATION OF PROTEINS (Z369314)
3. A PRACTICAL GUIDE TO PROTEIN AND PEPTIDE PURIFICATION FOR MICROSEQUENCING (Z350346)
4. HPLC OF MACROMOLECULES: A Practical Approach (H3409)

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