Product Information

N-Succinyl-Ala-Ala-Ala-*p*-nitroanilide

Elastase substrate

S4760

Product Description

CAS Number: 52299-14-6

Synonyms: *N*-Succinyl-tri-L-alanine 4-nitroanilide, *N*-Succinyl-L-alanyl-L-alanyl-L-alanine 4-nitroanilide, Suc-Ala-Ala-Ala-pNA, Suc-A-A-A-pNA, Suc-Ala3-pNA

Molecular Weight: 451.43

Molecular Formula: $C_{19}H_{25}N_5O_8$

The peptide *N*-Succinyl-Ala-Ala-Ala-*p*-nitroanilide (Suc-Ala-Ala-Ala-pNA) is routinely used to assay elastase activity.[1,2] This assay is highly reproducible and like many colorimetric assays, can be run in thirty minutes or less. Older and more complicated assays used another substrate, Elastin-Orcein (Catalogue Number E1500). The conversion factor for pancreatic elastase activity using these two substrates may be stated as follows:

1 Suc-Ala_3 unit ~ 30 Elastin-Orcein units

The buffers and solvents used in these assays can affect the substrate results.[3] Suc-Ala-Ala-Ala-pNA may be used for assays of other enzymes, such as alkaline proteases.[4]

Several publications,[5-8] theses,[9,10] and dissertations[11-20] have cited use of product S4760 in their protocols.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

This product should be stored at 2-8 °C.

Solubility

This product is tested for solubility in DMF at 25 mg/mL.

Various publications cite preparation of stock solutions of this material in DMSO, at 10 mM[18] and at 200 mM,[19] although we have not tested solubility in DMSO ourselves. Stock solutions of this material in DMSO may be stored in aliquots at −20 °C, although we have not ourselves tested solution stability of this material.

Usage

This product has been used as a substrate to determine the activity of leukocyte elastase.[2] In this assay:

- 25 µL of the enzyme solution was added to 1.8 mL of the substrate solution in 0.1 M HEPES buffer (pH 7.5), containing 0.5 M NaCl and 10% DMSO at 25 °C.
- The rate of substrate hydrolysis (the formation of *p*-nitroaniline) was measured at 410 nm at pH 7.5.

References

1. Bieth, J. *et al*., *Biochem. Med*., **11(4)**, 350-357 (1974).
2. Nakajima, K. *et al*., *J. Biol. Chem*., **254(10)**, 4027-4032 (1979).
3. Lestienne, P., and Bieth, J.G., *J. Biol. Chem*., **255(19)**, 9289-9294 (1980).
4. Bakhtiar, S. *et al*., *Enzyme and Microbial Technology*, **37(5)**, 534-540 (2005).
5. Stein, R.L., *J. Am. Chem. Soc*., **105(15)**, 5111-5116 (1983).

6. Boyd, D.W., Jr. *et al*., *Ann. Entomol. Soc. Am*., **95(3)**, 395-401 (2002).
7. Boyd, D.W., Jr., *Ann. Entomol. Soc. Am*., **96(5)**, 667-671 (2003).
8. Choi, H.-J. *et al*., *Korean J. Plant Res*., **32(3)**, 207-219 (2019).
9. Young, Mason, "Microdialysis Studies Using Porcine Pancreatic Elastase to Guide Mathematical Modeling of Microdialysis Sampling for *in vivo* Measurements". University of Arkansas, B.Sc.Eng. honors thesis, p. 11 (2016).
10. Pallister, Edward G., "Enzymatic Remodelling of Glycans & Glycan Analysis". University of Manchester, M.Sc. thesis, p. 59 (2020).
11. Perry, William Bryon, "Global Transcriptional Analysis of an *Escherichia coli* Recombinant Protein Process during Hypoxia and Hyperoxia". Massachusetts Institute of Technology, Ph.D. dissertation, p. 55 (2004).
12. Christen, Jayne M., "Analysis of Hemolymph Proteinase 16 and Serpin-3 from the Hemolymph of *Manduca Sexta*". Kansas State University, Ph.D. dissertation, p. 64 (2011).
13. Brugger, Daniel, "Experimental modelling of subclinical zinc deficiency in weaned piglets". Technische Universität München, Dr. agr. dissertation, p. 92 (2018).
14. Bryan, Dervan Dale Shian Lasien, "Characterization of protein sources and their effects on broiler performance, digestive tract morphology and caecal fermentation metabolites". University of Saskatchewan, Ph.D. dissertation, pp. 41, 68 (2018).
15. Pastorino, Giulia, "Evaluation of fodder legumes for nutraceutical, pharmaceutical and cosmetic applications". Università degli Studi di Genova, Ph.D. dissertation, pp. 29, 37, 44 (2018).
16. Zakaria, Nik Nur Azwanida Binti, "Evaluation of anti-ageing properties of *Moringa oliefera* Lam., *Centella asiatica* (L.) Urban, *Clitoria ternatea* L. and *Cosmos caudatus* Kunth. for potential application as cosmeceuticals". Newcastle University, Ph.D. dissertation, p. 106 (2019).
17. Roudnický, Pavel, "Molecular and Biochemical Characterization and Localization of Peptidase Inhibitors from *Eudiplozoon Nipponicum*". Masaryk University, Ph.D. dissertation, p. 78 (2021).
18. Abrahamse, Evan, "Protein digestion kinetics: a proxy for postprandial amino acid responses". Wageningen University, Ph.D. dissertation, p. 140 (2022).
19. Rajput, R. *et al*., *Enzyme Res*., **2010**, 132148 (2010).
20. Gradišar, H. *et al*., *Appl. Environ. Microbiol*., **71(7)**, 3420-3426 (2005).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

S4760pis Rev 07/22 CMH,RXR,GCY