Product Information

Peroxidase from horseradish

Sigma Type I, essentially salt-free, lyophilized powder, ≥50 units/mg solid (using pyrogallol)

P8125

Product Description

EC Number: 1.11.1.7
CAS Registry Number: 9003-99-0
Synonym: Hydrogen peroxide oxidoreductase; HRP

Horseradish peroxidase (HRP) is isolated from the roots of horseradish (*Amoracia rusticana*) and belongs to the ferroprotoporphyrin group of peroxidases. HRP readily combines with hydrogen peroxide (H_2O_2). The resultant [HRP-H_2O_2] complex can oxidize a wide variety of hydrogen donors:

Donor + H_2O_2 → Oxidized Donor + 2 H_2O

HRP will oxidize various substrates (see Table 1):

- Chromogenic
- Chemiluminescent (such as luminol or isoluminol)
- Fluorogenic (such as tyramine, homovanillic acid, or 4-hydroxyphenyl acetic acid)

HRP is a single chain polypeptide that contains four disulfide bridges. HRP is a glycoprotein that contains 18% carbohydrate. The carbohydrate composition consists of galactose, arabinose, xylose, fucose, mannose, mannosamine, and galactosamine, depending upon the specific isozyme.[1]

HRP is a widely used label for immunoglobulins in many different immunochemistry applications, including immunoblotting, immunohistochemistry, and ELISA. HRP can be conjugated to antibodies by several different methods, including glutaraldehyde, periodate oxidation, through disulfide bonds, and also via amino and thiol directed cross-linkers. HRP is the most desired label for antibodies, since it is the smallest and most stable of the three most popular enzyme labels (peroxidase, β-galactosidase, alkaline phosphatase) and its glycosylation leads to lower non-specific binding.[2] A review of glutaraldehyde and periodate conjugation methods has been published.[3]

Peroxidase is also utilized for the determination of glucose[4] and peroxides[5] in solution. Several publications,[6-25] theses,[26-35] and dissertations[36-44] have cited use of P8125 in their research 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.

Reagent

This product is supplied as an essentially salt-free, lyophilized powder.

Specific Activity: ≥ 50 units/mg solid (pyrogallol as substrate)

Unit definition (purpurogallin): One unit will form 1.0 mg of purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20 °C. This unit is equivalent to ~18 µM units per minute at 25 °C.

RZ (Reinheitszahl): ≥ 1.0

RZ is the absorbance ratio A_{403}/A_{275} determined at 0.5-1.0 mg/mL in deionized water. RZ is a measure of hemin content, **not** enzymatic activity. Even preparations with high RZ values may have low enzymatic activity.

Total molecular mass:[45] ~44 kDa (~44,000 Da)

- Polypeptide chain: 33,890 Da
- Hemin plus Ca^{2+}: ~700 Da
- Carbohydrate: 9,400 Da

Extinction coefficient:[46] E^{mM} = 100 (403 nm)

Optimal pH range:[47] 6.0-6.5 (activity at pH 7.5 is 84% of the maximum)

The enzyme is most stable in the pH range of 5.0-9.0.

Isoelectric point:[1] isozymes range from 3.0-9.0 (at least seven isozymes)

Inhibitors:[48] sodium azide; cyanide; L-cystine; dichromate; ethylenethiourea; hydroxylamine; sulfide; vanadate; *p*-aminobenzoic acid; Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+} ions

Preparation Instructions

This product is soluble in water or 0.1 M phosphate buffer, pH 6.0.

Storage/Stability

Store the product at 2-8 °C. The enzyme remains active for at least two years. Solutions show a loss of <2% of activity per week if stored at -20 °C.

References

1. Shannon, L.M. *et al*., *J. Biol. Chem*., **241(9),** 2166-2172 (1966).
2. Deshpande, S.S., *Enzyme Immunoassays, From Concept to Product Development*. Chapman and Hall (New York, NY), pp. 169-171 (1996).
3. Harlow, E. and Lane, D., *Antibodies: A Laboratory Manual*. Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY), pp. 346-348 (1988).
4. Bergmeyer, H.-U. *et al*., *Methods of Enzymatic Analysis*, 2nd ed. (Bergmeyer, H.-U., ed.). Verlag Chemie/Academic Press, pp. 1205-1227 (1974).
5. Bernt, E. and Bergmeyer, H.-U., *Methods of Enzymatic Analysis*, 2nd ed., pp. 2246-2248 (1974).
6. Otten, S., and Rosazza, J.P., *Appl. Environ. Microbiol.*, **35(3)**, 554-557 (1978).
7. Zhang, Z.. *et al.*, *Plant J.*, **8(1)**, 139-145 (1995).
8. Black, M. *et al*., *J. Exp. Bot.*, **47(295)**, 161-169 (1996).
9. Wyka, T., *Oecologia,* **120(2)**, 198-208 (1999).
10. Tedjo, W. *et al*., *J. Food Sci*., **65(8)**, 1284-1287 (2000).
11. Audi, S.H. *et al*., *Am. J. Physiol. Lung Cell. Mol. Physiol*., **285(5)**, L1116-1131 (2003).
12. Leterme, P., and Théwis, A., *Reprod. Nutr. Dev.*, **44(5)**, 407-417 (2004).
13. Sanz, V. *et al*., *J. Am. Chem. Soc*., **127(3)**, 1038-1048 (2005).
14. Calligaris, S. *et al.*, *Pediatr. Res.*, **62(5)**, 576-580 (2007).
15. Hiyane, R. *et al.*, *Ann. Bot.*, **106(3)**, 395-403 (2010).
16. Hong, J.-S. *et al*., *J. Immunol.*, **186(11)**, 6427-6436 (2011).
17. Marchesini, G. *et al*., *Ital. J. Animal Sci.*, **11(2)**, e37 (2012).
18. Zhao, Z. *et al*., *J. Mater. Chem. B*, **1(37)**, 4719-4722 (2013).
19. García-Ayuso, D. *et al*., *Br. J. Opthamol.*, **98(3)**, 396-401 (2014).
20. Larson-Casey, J.L., and Carter, A.B.., *Bio. Protoc.*, **6(22)**, e2010 (2016).
21. Voothuluru, P. *et al*., *Plant. Physiol.*, **178(3)**, 1002-1010 (2018).
22. Hüttner, S. *et al*., *Appl. Environ. Microbiol.*, **85(23)**, e01408-01419 (2019).
23. Cacciottola, L. *et al*., *F&S Science*, **2(2)**, 141-152 (2021).
24. Mazorra-Carrillo, J.L *et al*., *Front. Vet. Sci.*, **8**, 734087 (2021).
25. Hatakeyama, D.M. *et al*., *PLoS One*, **16(6)**, e0253050 (2021).
26. Grubb, Robert Thomas, "Effects of *Brachypterolus pulicarius* on growth and seed production of Dalmation toadflax". Montana State University, M.S. thesis, p. 72 (1998).
27. Wilson, Cindy, "Hydrogen Peroxide Diel Cycling in Geothermal Waters: Photochemical Formation Through Metal Redox Cycling". University of Montana, M.S. thesis, p. 14 (1998).
28. Paz, Maria del Pilar, "Rhizome manipulation affects growth and development of ornamental gingers". Louisiana State University, M.S. thesis, p. 16 (2003).
29. Roets, Nicolaas J.R., "Factors influencing the occurrence of premature and excessive leaf abscission in the avocado (*Persea americana mill*.) cultivar 'Ryan' and possible preventative measures". University of Pretoria, M.Sc. thesis, p. 85 (August 2009).
30. Burden, Petro Trudene, "The effect of endosperm vitreousness on fermentation characteristics and *in vitro* digestibility of maize". Stellenbosch University, M.Sc. thesis, p. 57 (2010).
31. Ribeiro, Anne Luise, "Effects of North Sea Oil on Biotransformation and Immune Responses in Juvenile Atlantic Cod (*Gadus morhua*)". University of Oslo, M.Sc. thesis, p. 65 (2014).
32. Pomposini, Amanda Nicole, "Biology and Impacts of Leech Infestation in Largemouth Bass (*Micropterus Salmoides*) in Back Bay, Virginia". Old Dominion University, M.S. thesis, p. 24 (2017).
33. Branstrom, Isabel Anne, "Postharvest cold storage affects flower life and physiology of potted tulips". Cornell University, M.S. thesis, p. 23 (2018).
34. Yonke, Joseph A., "Effects of High-docosahexaenoic Acid Microalgae and Choline in the Hen Diet on Egg Production and Lipid Metabolism". Oregon State University, M.S. thesis, p. 55 (2018).

35. Blakstad, Jakob Irgens, "The utilization of frass from the yellow mealworm (*Tenebrio molitor*) as a plant fertilizer and immune stimulant". Norwegian University of Science and Technology, M.Sc. thesis, p. 29 (May 2021).
36. Christiansen, Jesper Valentin, "The behaviour of iodine in the terrestrial environment. An investigation of the possible enzymatically controlled iodination of humic acid". Risø National Laboratory (Denmark), Ph.D. dissertation, p. 134 (1990).
37. Schooneveld-Bergmans, M.E.F., "Wheat bran glucuronoarabinoxylans: biochemical and physical aspects". Wageningen University, Ph.D. dissertation, p. 72 (1997).
38. Wu, Danu, "Investigation of conjugated polyelectrolytes and their protein sensing by fluorescence correlation spectroscopy". University of Florida, Ph.D. dissertation, p. 138 (2012).
39. Ellam, Samantha Lee, "Cocoa flavanol interactions with intestinal sugar metabolism". University of Leeds, Ph.D. dissertation, p. 58 (March 2013).
40. Hoehl, Melanie Margarete, "Versatile, automated sample preparation and detection of contaminants and biological materials". Massachusetts Institute of Technology, Ph.D. dissertation, p. 35 (2013).
41. Ingold, Irina, "Interrogating the *in vivo* significance of selenium-based catalysis of GPX4 for mammalian development and survival". Technischen Universität München, Dr. rer. nat. dissertation, p. 25 (2014).
42. Dong, Wen, "Extensin Peroxidase Identification and Characterization in *Solanum lycopersicum*". Ohio University, Ph.D. dissertation, p. 53 (2015).
43. Rawat, Vipin Singh, "DNA damage induction by bilirubin induced oxidative stress and activation of DNA repair pathways by bilirubin". The Open University, Ph.D. dissertation, p. 48 (2018).
44. Singha, Prosanta Kumar, "Development of methods and inhibitors to target key enzymatic nodes generating protumorigenic lipid signaling". University of Eastern Finland, Ph.D. dissertation, p. 41 (2020).
45. Welinder, K.G., *Eur. J. Biochem*., **96(3),** 483-502 (1979).
46. Delincée, H. and Radola, B.J., *Eur. J. Biochem*., **52(2),** 321-330 (1975).
47. Schomberg, D., Salzmann, M., and Stephan, D., *Enzyme Handbook 7*, EC 1.11.1.7, 1-6 (1993).
48. Zollner, H., *Handbook of Enzyme Inhibitors*, 2nd ed., Part A. VCH Verlagsgesellschaft, pp. 367-368 (1993).

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Table 1. Peroxidase Substrates

Substrate	Cat. No. or Cat. Nos.	Color Reaction	End Product	Applications
2,2′-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid; ABTS)	A3219, A9941	**Green**	Soluble	ELISA
o-Phenylenediamine (OPD)	P9187	**Orange**	Soluble	ELISA
3,3′,5,5′-Tetramethylbenzidine (TMB)	T8665, T3405	**Blue**	Soluble	ELISA
	T0565	**Deep Blue**	Insoluble	Blotting
o-Dianisidine	D9154	**Yellow-Orange**	Soluble	ELISA
5-Aminosalicylic Acid (5AS)	A79809, A3537	**Brown**	Soluble	ELISA
3,3′-Diaminobenzidine (DAB)	D7304, D5905, D4168, D4293, D4418, D7679	**Brown**	Insoluble	Blotting, Histochemistry
	D0426	**Blue-Black**		
4-Chloro-1-Naphthol (4C1N)	C6788	**Blue**	Insoluble	Blotting
3-Amino-9-Ethylcarbazole (AEC)	AEC101, A6926	**Red**	Insoluble	Blotting
CPS-1	CPS160, CPS1A120, CPS1A300	Chemiluminescent	Soluble	Blotting
CPS-3	CPS350, CPS3100, CPS3500			
CPS-2	CPS260	Chemiluminescent	Soluble	ELISA

Table 2. Other Grades of HRP Available

Cat. No.	RZ value	Specific Activity (*)
P8250	≥ 1.8	150 – 250 units/mg solid
P2088	2.6 – 3.4	200 – 300 units/mg solid
P8415	≥ 3.0	≥ 250 units/mg solid
P8375	2.5 – 4.0	≥ 250 units/mg solid
P6782	2.5 – 4.0	≥ 250 units/mg solid
P6140	2.5 – 3.5	≥ 225 units/mg protein

(*) Specific activity is reported in terms of purpurogallin units.

P8125dat Rev 02/22 RBG,SM,CS,MAM,GCY