Product Information

Peroxidase from horseradish

Sigma Type II, essentially salt-free, lyophilized powder, 150-250 units/mg solid (using pyrogallol)

P8250

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 theses[6-8] and dissertations[9-14] have cited use of P8250 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: 150-250 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): ≥ 1.8

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:[15] ~44 kDa (~44,000 Da)

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

Extinction coefficient:[16] E^{mM} = 100 (403 nm)

Optimal pH range:[17] 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:[18] 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 2 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. de Mooy, Anthony Brett, "Mechanism and subpopulation specificity of mitochondrial reactive oxygen species release in the post-ischemic hyperthyroid myocardium". Colorado State University, M.S. thesis, p. 26 (2012).
7. Buchholz, Martina Irene, "Increased Stabiliity and Activity of Alcohol Oxidase Under High Hydrostatic Pressure". University of Georgia, M.S. thesis, p. 34 (2016).
8. Hubert, Matthew, "Investigating the Effects of 2-Aminoadipic Acid on Beta-Cell and Human Islet Function". University of Alberta, M.Sc. thesis, p. 14 (2020).
9. Kim, Dong-Beon, "Production and Decomposition of Hydrogen Peroxide by Marine Phytoplankton". Old Dominion University, Ph.D. dissertation, p. 166 (1993).
10. Hartman, Angela D., "Effect of Metabolic Enzymes on Amylopectin Content and Infectivity of *Cryptosporidium parvum*". Virginia Polytechnic Institute and State University, Ph.D. dissertation, p. 45 (2006).
11. Annan, Bernard Derek, "Optical Modulation of High-Affinity Biomolecules Function via Photochromic Dyes: A Step towards an Artificial Control of Biological Activity". Cranfield University, Ph.D. dissertation, p. 93 (2008).
12. Bharadwaj, Vimala Nagabhushana, "Comprehensive Assessment of Nanoparticle Delivery after Experimental Traumatic Brain Injury". Arizona State University, Ph.D. dissertation, pp. 23, 46, 65 (2018).
13. Pelili, Maria, " NADPH Oxidase and Xanthine Oxidoreductase as Targets and Regulators of the Nitrate-Nitrite-Nitric Oxide Pathway". Karolinska Institute, Ph.D. dissertation, p. 31 (2018).
14. Christensen, David George, "Regulation of Acetyl Phosphate-Dependent Acetylation and Identification of Novel Lysine Acetyltransferases in *Escherichia coli*". Loyola University, Ph.D. dissertation, p. 61 (March 2019).
15. Welinder, K.G., *Eur. J. Biochem*., **96(3),** 483-502 (1979).
16. Delincée, H. and Radola, B.J., *Eur. J. Biochem*., **52(2),** 321-330 (1975).
17. Schomberg, D., Salzmann, M., and Stephan, D., *Enzyme Handbook 7*, EC 1.11.1.7, 1-6 (1993).
18. 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 (*)
P6782	2.5 – 4.0	≥ 250 units/mg solid
P2088	2.6 – 3.4	200-300 units/mg solid
P8415	≥ 3.0	≥ 250 units/mg solid
P8125	≥ 1.0	50-150 units/mg solid
P8375	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.

P8250dat Rev 02/22 RBG,CS,MAM,GCY