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Product Information

Peroxidase from horseradish
Sigma Type I

Catalog Number **P8125**
Storage Temperature 2–8 °C

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

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

$$\text{Donor} + H_2O_2 \rightarrow \text{Oxidized Donor} + 2\ H_2O$$

Peroxidase will oxidize a variety of substrates (see Table 2): chromogenic, chemiluminescent (luminol and isoluminol), and fluorogenic (tyramine, homovanillic acid, and 4-hydroxyphenyl acetic acid).

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

Total molecular mass:[3] ~44 kDa
polypeptide chain: 33,890 Da
hemin plus Ca^{2+}: ~700 Da
carbohydrate: 9,400 Da

Extinction coefficient:[2] E^{mM} = 100 (403 nm)

Optimal pH range:[5] 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:[4] sodium azide, cyanide, L-cystine, dichromate, ethylenethiourea, hydroxylamine, sulfide, vanadate, *p*-aminobenzoic acid, and Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, and Pb^{2+} ions

HRP is a widely used label for immunoglobulins in many different immunochemistry applications, including ELISA, immunoblotting, and immunohistochemistry. 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 (HRP, β-galactosidase, and alkaline phosphatase), and its glycosylation leads to lower non-specific binding.[6] A review of glutaraldehyde and periodate conjugation methods has been published.[7]

Peroxidase is also utilized for the determination of glucose[8] and peroxides[9] in solution.

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

Specific Activity: 50–150 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: ≥1.0
RZ (Reinheitszahl) is the absorbance ratio A_{403}/A_{275} determined at 0.5–1.0 mg/ml in deionized water. It is a measure of hemin content, not enzymatic activity. Even preparations with high RZ values may have low enzymatic activity.

Precautions and Disclaimer
This product is 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.

Preparation Instructions
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.

Related Products

Table 1.
Other Grades of HRP available

Catalog Number	RZ value	Specific Activity (*)
P6782	~3.0	250–330 units/mg solid
P2088	~3.0	200–300 units/mg solid
P8415	≥3.0	≥250 units/mg solid
P8375	~3.0	≥250 units/mg solid
P8250	≥1.8	150–250 units/mg solid
P6140	2.5–3.5	≥225 units/mg protein

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

References

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3. Welinder, K.G., *Eur. J. Biochem*., **96(3)**, 483-502 (1979).
4. Zollner, H., *Handbook of Enzyme Inhibitors*, 2nd Ed., Part A: 367-368 (1993).
5. Schomberg, D., Salzmann, M., and Stephan, D., *Enzyme Handbook 7*, EC 1.11.1.7:1-6 (1993).
6. Deshpande, S.S., *Enzyme Immunoassays, From Concept to Product Development*, Chapman and Hall, 169-171 (1996).
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8. Bergmeyer, H.U. *et al*., *Methods of Enzymatic Analysis* (Bergmeyer, H.U., ed.), pp. 1205-1227 (1974).
9. Bernt, E., and Bergmeyer, H.U., *Methods of Enzymatic Analysis* (Bergmeyer, H.U., ed.), pp. 2246-2248 (1974).

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Table 2.
Peroxidase Substrates

Substrate	Catalog Number	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-Dianisdine	D9154	Yellow-Orange	Soluble	ELISA
5-Aminosalicylic Acid (5AS)	A6178	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 CPS1120 CPS1300	Chemiluminescent	Soluble	Blotting
CPS-3	CPS350 CPS3100 CPS3500			
CPS-2	CPS260 CPS2120 CPS2300	Chemiluminescent	Soluble	ELISA