MP Biomedicals, LLC
29525 Fountain Parkway
Solon, Ohio 44139
Telephone: 440/337-1200
Toll Free: 800/854-0530
Fax: 440/337-1180
mailto: biotech@mpbio.com
web: http://www.mpbio.com

TECHNICAL INFORMATION

Catalog Number: 150036, 150037, 150038, 191398, 195371, 195372, 195373, 195374
Peroxidase

Molecular Weight: Approximately 44,000 (Polypeptide = 33,890; hemin + calcium = approximately 700; carbohydrate = approximately 9365).[31]

CAS # 9003-99-0

Synonyms: HRP; Donor:hydrogen-peroxide oxidoreductase; **E.C.** 1.11.1.7

Physical Description: Tan crystalline powder

Source: Horseradish (*Amoracia rusticana*)

RZ Definition: The ratio of the absorbance at 403 nm to the absorbance at 275 nm. This value is an expression of the ratio of hemin to protein content.

Optimum pH: 7.0

Isoelectric point: 7.2

Inhibitors: HRP is reversibly inhibited by cyanide and sulfide at a concentration of 10^{-5} M. The following can also inhibit HRP: p-Aminobenzoic acid, azide, cyclopropanone, L-cystine, dichromate, ethylenethiourea, hydroxylamine, sulfite and vanadate and the divalent metals Cd, Co, Cu, Fe, Mn, Ni, Pb.[30]

Specificity: Activity is observed with H_2O_2, MeOOH and EtOOH.

Solubility: Soluble in distilled water. Soluble in 0.1 M potassium phosphate buffer, pH 6.0 (monobasic potassium phosphate adjusted to pH 6.0 with 1.0 M potassium hydroxide) (10 mg/ml yields a clear, red-brown solution).

Solutions of HRP can be kept refrigerated for up to a year while maintaining practically undiminished activity.

Description: HRP is a plant glycohemoprotein. The enzymatic activity of HRP is due to the cyclic reduction and oxidation of the iron atom in the hematin group. The iron-containing hemin group is covalently bound to the glycoprotein apoenzyme. HRP readily combines with hydrogen peroxide (H_2O_2) and the resultant [HRP·H_2O_2] complex can oxidize a wide variety of chromogenic hydrogen donors.[15]

HRP is composed of seven isozymes.[22] All isozymes contain photohemin IX as prosthetic group. Neutral and amino sugars account for approximately 18% of the enzyme. The active site of HRP involves both the apoprotein and the heme group.[32]

Use: Peroxidase has use in immunochemistry. HRP labeled immunoglobulins are used as probes for the demonstration of tissue antigens and in enzyme immunoassay (EIA) systems for determination of soluble and insoluble antigens.[3,6,21]

It is also useful for tracing neural connections. These include motor and sensory innervation of peripheral organs, and connections of peripheral nerves, ganglia and individual dorsal roots.[15]

Substrate System	Color Reaction	End Product	Applications
2,2'-Azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS)	Green	Soluble	ELISA
o-Phenylenediamine (OPD)	Orange	Soluble	ELISA
3,3',5,5'-Tetramethylbenzidine (TMB)	Blue	Soluble	ELISA
o-Dianisidine	Yellow- Orange	Soluble	ELISA
5-Aminosalicylic Acid (5AS)	Brown	Soluble	ELISA

3,3'-Diaminobenzidine (DAB)	Brown	Soluble	Immunoblotting Immunohistochemistry
3-Amino-9-Ethylcarbazole (AEC)	Red	Insoluble	Immunoblotting Immunohistochemistry
4-Chloro-1-naphthol (4C1N)	Blue	Insoluble	Immunoblotting

Activity Assay:[5]

Assay Method: The method of assay in which the rate of decomposition hydrogen peroxidide by peroxidase with pyrogallol as the hydrogen donor is determined spectrophotometrically by measuring the rate of color development at 420 nm.

$$H_2O_2 + \text{Pyrogallol (donor)} \xrightarrow{\text{Peroxidase}} 2H_2O + \text{Purpurogallin (oxidized donor)}$$

Unit Definition: That amount of enzyme which catalyses the production of one milligram of purpurogallin in 20 seconds at 20°C. at a pH of 6.0. The purpurogallin (20 sec.) unit is equivalent to approximately 18 uM units per minute at 25°C.

Reagents:

A. 100 mM Potassium phosphate buffer, pH 6.0 at 20°C: Prepare 100 ml in deionized water using potassium phosphate, monobasic, anhydrous. Adjust to pH 6.0 at 20°C with 1.0 M KOH.
B. 0.50% (w/w) Hydrogen peroxide solution (H_2O_2): Prepare 50 ml in deionized water using a 30% Hydrogen peroxide solution. **Prepare fresh each time.**
C. 5% (w/v) Pyrogallol Solution: Prepare 10 ml in deionized water using pyrogallol. **Prepare fresh for each use. Protect from light.**
D. Peroxidase Enzyme Solution: Immediately before use, prepare a solution containing 0.4 to 0.7 unit/ml of peroxidase in cold Reagent A.

Procedure:

Pipette the following reagents into suitable cuvettes:

	Test	**Blank**
Deionized Water	2.10 ml	2.10 ml
Reagent A	0.32 ml	0.32 ml
Reagent B	0.16 ml	0.16 ml
Reagent C	0.32 ml	0.32 ml

Mix by inversion and equilibrate to 20°C. Monitor the A_{420nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent A	--	0.10 ml
Reagent D	0.10 ml	--

Immediately mix by inversion and record the increase in $A_{420\ nm}$ for approximately 5 minutes. Obtain the DA_{420nm}/20 seconds using the maximum linear rate (the enzyme concentration may have to be modified in order for the rate, DA_{420nm}/20 seconds, to be within the specified range of 0.16-0.28) for both the Test and Blank.

Final Assay Concentrations: In a 3.00 ml reaction mix, the final concentrations are 14 mM potassium phosphate, 0.027% (w/w) hydrogen peroxide, 0.5% (w/v) pyrogallol and 0.04-0.07 unit peroxidase.

Calculation:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{420nm}/20 \text{ second Test} - \Delta A_{420nm}/20 \text{ second Blank})(3)(df)}{(12)(0.1)}$$

Where:

3 = Volume (in milliliters) of assay
df = Dilution factor
12 = Extinction coefficient of 1 mg/ml of purpurogallin at 420 nm
0.1 = Volume (in milliliters) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

RZ Determination:

– Prepare a 0.5 mg/ml solution of horseradish peroxidase. Weigh out approximately 5 mg of peroxidase and dissolve in a volume of saline to give a 0.5 mg/ml solution.
– Using saline as a blank, read the absorbance of the peroxidase solution at 403 nm and 275 nm.
– Divide the absorbance at 403 nm by that at 275 nm to obtain the Rz value.

Availability:

Catalog Number	Description	Size
150036	HRP, salt-free, lyophilized, RZ > 0.60; Activity: approximately 60 units/mg solid	5 KU 10 KU 25 KU 50 KU
150037	HRP, salt-free, lyophilized, RZ = 1.0-2.0; Activity: approximately 100-150 units/mg solid	5 KU 10 KU 25 KU 50 KU
150038	HRP, salt-free, lyophilized; RZ > 3.0; Activity: approximately 300 units/mg solid	5 KU 10 KU 25 KU 50 KU
191398	HRP, lyophilized powder, conjugation grade, activated; Activity approximately 3000 units/ml after reconstitution with distilled water. An activated form of HRP ready for conjugation. No additional preparation is necessary. 1 mg of activated HRP should yield 0.5 ml of conjugate, working dilution 1:2000	500 ug 1 mg 5 mg
195371	HRP, essentially salt-free, lyophilized; Acitivity: approximately 80 units/mg solid	5 KU 25 KU 50 KU 100 KU 200 KU
195372	HRP, essentially salt-free, lyophilized, RZ = 1.0 to 1.5; Activity approximately 150-200 units/mg solid	5 KU 25 KU 50 KU 100 KU 200 KU
195373	HRP, essentially salt-free, lyophilized; Activity approximately 250-330 units/mg solid	1 KU 2 KU 5 KU 10 KU 25 KU 10^5 KU
195374	HRP, crystalline suspension in 3.2 M ammonium sulfate with potassium phosphate buffer, pH 6.0; RZ = ~3.0; Activity approximately 250 units/mg protein	2 KU 5 KU 10 KU 25 KU

References:

– de Aspuru, E.O. and Zaton, A.M.L., "Effect of glutathione on horseradish peroxidase activity." *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **v. 55:11**, 2343-2346 (1999).
– Avrameas, S. and Ternynck, T., "Peroxidase Labelled Antibody and Fab Conjugates with Enhanced Intracellular Penetration," *Immunohistochemistry*, **Vol. 8**, 1175-1179, (1971).
– Beesley, J.E. (ed.), *Immunocytochemistry: A Practical Approach*, IRL Press at Oxford University Press, Oxford England (1993).
– Bergmeyer, H.U., *Methods of Enzymatic Analysis* 1, 495, 2nd. ed., (1974).
– Chance, B. and Maehly, A.C., *Methods in Enzymology*, **v. II**, 773-775 (1955).
– Cuello, A.C., *Immunohistochemistry*, **II**, John Wiley & Sons, Ltd., Chichester, England (1993).
– Gosling, J.P., "Enzyme Immunoassay: With and Without Separation." *Principles and Practice of Immunoassay*, *2nd. edition*, Eds. Price, C.P. and Newman, D.J. MacMillan, London, (1997).
– Haqqani, A.S, Sandhu, J.K. and Birnboim, H.C., "A myeloperoxidase-specific assay based upon bromide-dependent chemiluminescence of luminol." *Anal. Biochem.*, **v. 273:1**, 126-132 (1999).
– Hewson, W.D., Hager, L.P., *The Prophyrins*, **v. 7**, 295 (1979).
– Imagawa, M., Yoshitake, S., Hamaguchi, Y., Ishikawa, E., Nitsu, Y., Urushizaki, I., Kanazawa, R., Tachibena, S., Nazakawa, N., and Ogawa, "Characteristics and Evaluation of Antibody-Horseradish Peroxidase Conjugates Prepared by Using a Maleimide Compound, Glutaraldehyde, and Periodate." *Journal of Applied Biochemistry*, **v. 4**, 41-57, (1982).
– Kitagawa, T. et al., "Enzyme Immunoassay of Blasticidin S with High Sensitivity: A New and Convenient Method for Preparation of Immunogenic Conjugates," *J. Biochem.*, **v. 92**, (1982).
– Kitagawa, T., et al., "Preparation and Characterisation of Hetero-bifunctional Cross-Linking Reagents for Protein Modifications," *Chem. Pharm. Bull.*, **v. 29(4)**, 1130-1135, (1981).
– Maehly, A.C., *Plant Peroxidase. Methods in Enzymology, Vol. II*, ed. Colowick and Kaplan, 807, (Academic Press, New York).
– Masuho, Y., Kishida, K., Saito, M., Umemoto, N., and Hara, T. "Importance of the Antigen Binding Valency and the Nature of the Cross-Linking Bond in Ricin A- Chain Conjugates with Antibody." *J. Biochem.*, **v. 91**, 1583-1591, (1982).
– Mesulam, M. (ed.), *Tracing the Neural Connections with Horseradish Peroxidase*, John Wiley & Sons, New York (1982).
– Najbauer, J., Vecsei, L. and Palffy, G., "Non-specific peroxidase (donor:H2O2-oxidoreductase, EC 1.11.1.7) activity in multiple sclerosis." *Acta Med. Hung.*, **v. 47:3-4**, 129-133 (1990).
– Nakane, P.K. and Kawaoi, A., "Peroxidase-Labelled Antibody: A New Method of Conjugation." *Journal of Histochemistry and Cytochemistry*, **Vol. 22, No. 12**, 1084-1091, (1974).
– Olsen, R.L., Little, C., *Biochem. J.*, **v. 222**, 701 (1984).
– O'Sullivan, M.J., Gnemmi, E., Morris, D., Chieregatti, G., Simmonds, A.D., Simmons, M., Bridges, J.W., and Marks, V., "Comparison of Two Methods of Preparing Enzyme-Antibody Conjugates: Application of These Conjugates for Enzyme for Enzyme Immunoassay," *Analytical Biochemistry*, **v. 100**, 100-108, (1979).
– Paul, K.G., *The Enzymes, 2nd Ed.*, v. 8, 227 (1963).
– Roitt, I.M., *Immunology, 3rd Ed.*, Mosby-Year Book Europe Limited, London, UK (1993).
– Shannon et al., *J. Biol. Chem.*, **v. 241**, 2166, (1966).
– Shinmen, Y., et al., *Agric. Biol. Chem.*, **v. 50**, 247 (1986).
– Stiborova, M., Schmeiser, H.H. and Frei, E., "Oxidation of xenobiotics by plant microsomes, a reconstituted cytochrome P450 system and peroxidase: a comparative study." *Phytochemistry*, **v. 54:4**, 353-362 (2000).
– Sumner, J.B. and Gjessing, E.C., *Arch. Biochem.*, **v. 2**, 1291, (1943).
– Tijssen, P. and Kurstak, E., "Highly Efficient and Simple Methods for the Preparation of Peroxidase and Active Peroxidase-Antibody Conjugates for Enzyme Immunoassays." *Anal. Biochem.*, **v. 136**, 451-457, (1984).
– Valoti, M., et al., "Evidence of a coupled mechanism between monoamine oxidase and peroxidase in the metabolism of tyramine by rat intestinal mitochondria." *Biochem. Pharmacol.*, **v. 55:1**, 37-43 (1998).
– Weltman, J.K., Johnson, S-A., Langevin, J. and Riester, E.F. *Biotechniques. N-Succinimidyl (4-Iodoacetyl) Aminobenzoate: A New Heterobifunctional Crosslinker*, 148-152.(1983).
– Yoshitake, S., Imagawa, M., and Ishikawa, E., "Efficient Preparation of Rabbit Fab-Horseradish Peroxidase Conjugates using Maleimide Compounds, and Its Use for Enzyme Immunoassay," *Analytical Letters*, **v. 15(BZ)**, 147-160, (1982).
– Zollner, H. (ed), *Handbook of Enzyme Inhibitors, Part A, 2nd Ed.*, VCH, Weinheim, Germany (1993).
– *European Journal of Biochemistry*, **v. 96**, 483 (1979).
– Weinryb, I., "Behavior of Horseradish Peroxidase at High Hydrogen Peroxide Concentrations." *Biochem.*, **v. 5**, 2003 (1966).