

Product Information

Peroxidase from horseradish

Sigma Type VI-A, essentially salt-free, lyophilized powder, 950-2000 units/mg solid (using ABTS), ≥ 250 units/mg solid (using pyrogallol)

P6782

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 (*Amaracia rusticana*) and belongs to the ferroporphyrin 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:

$$\text{Donor} + \text{H}_2\text{O}_2 \rightarrow \text{Oxidized Donor} + 2 \text{H}_2\text{O}$$

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 glucose⁴ and peroxides⁵ in solution. Several publications⁶⁻¹⁸ and dissertations¹⁹⁻⁴⁰ have cited use of P6782 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. Please note that Cat. No. P6782 is sold by weight (as MG, or milligrams), rather by enzyme units (such as KU, or kilounits).

Specific Activity:

- ≥ 250 units/mg solid (pyrogallol as substrate)
- 950-2,000 units/mg solid (ABTS as substrate)

Note: Using 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) tablets (Cat. No. A9941) as the substrate, $\sim 4\times$ the activity is observed when compared to pyrogallol (purpurogallin unit).

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 $\sim 18 \mu\text{M}$ units per minute at 25 °C.

RZ (Reinheitzzahl): 2.5 – 4.0

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:⁴¹ ~ 44 kDa ($\sim 44,000$ Da)

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

Extinction coefficient:⁴² $E_{\text{mM}} = 100$ (403 nm)Optimal pH range:⁴³ 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:⁴⁴ sodium azide; cyanide; L-cystine; dichromate; ethylenethiourea; hydroxylamine; sulfide; vanadate; *p*-aminobenzoic acid; Cd²⁺, Co²⁺, Cu²⁺, Fe³⁺, Mn²⁺, Ni²⁺, Pb²⁺ 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 five years. The product may also be stored at -20 °C, if desired. The lyophilized powder will retain at least 80% of its activity after two weeks at 37 °C.

A 1 mg/mL solution in 0.1 M phosphate buffer, pH 6.0, remains active for at least two weeks at room temperature. A solution retains activity after 5 freeze-thaw cycles.

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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
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.

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