Product Information

Peroxidase from horseradish

Sigma Type X, ammonium sulfate suspension

P6140

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,[6-10] theses,[11] and dissertations[12-16] have cited use of P6140 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 ammonium sulfate suspension.

Specific Activity: ≥ 225 units/mg protein (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): 2.5 – 3.5

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:[17] ~44 kDa (~44,000 Da)

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

Extinction coefficient:[18] E^{mM} = 100 (403 nm)

Optimal pH range:[19] 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:[20] 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

Water may be used to dilute the suspension if needed. The page "How to Work with Enzymes Supplied as Ammonium Sulfate Suspensions" is available at www.sigmaaldrich.com for additional general consulation.

Storage/Stability

Store the product at 2-8 °C. **DO NOT FREEZE**. We strongly recommend that ammonium sulfate suspensions **not** be frozen.

References

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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
P8125	≥ 1.0	≥ 50 units/mg solid

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

P6140dat Rev 02/22 RBG,SM,CS,MAM,GCY