

## Product Information

### PEROXIDASE FROM HORSERADISH

HRP

Product Numbers:

**P6782**, Type VI-A

**P8375**, Type VI

**P2088**, Type VI, Highly stabilized

**P8415**, Type XII, Affinity purified

**P8125**, Type I

**P8250**, Type II

**P6140**, Type X

CAS #: 9003-99-0

EC# 1.11.1.7

#### Product Description

Appearance: All products are red/brown powders with the exception of P6140 which is a brown suspension.

Extinction coefficient:  $E_{mM} = 100$  when measured at 403 nm.<sup>1</sup>

Horseradish peroxidase (HRP) is isolated from horseradish roots (*Aморacia rusticana*) and belongs to the ferroporphyrin group of peroxidases. 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.<sup>2</sup> Its molecular weight (approx. 44 kDa) includes the polypeptide chain (33,890 Daltons), heme plus  $Ca^{2+}$  (approx. 700 Daltons), and carbohydrate (9400 Daltons).<sup>3</sup> At least seven isozymes of HRP exist.<sup>2</sup> The isoelectric point for horseradish peroxidase isozymes ranges from 3.0 - 9.0.

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 (see table on page 2). It can also utilize chemiluminescent substrates such as luminol and isoluminol and fluorogenic substrates such as tyramine, homovanillic acid, 4-hydroxyphenyl acetic acid. The following compounds are inhibitors of horseradish peroxidase: sodium azide, cyanide, L-cystine, dichromate, ethylenethiourea, hydroxylamine, sulfide, vanadate, p-aminobenzoic acid,  $Cd^{+2}$ ,  $Co^{+2}$ ,  $Cu^{+2}$ ,  $Fe^{+3}$ ,  $Mn^{+2}$ ,  $Ni^{+2}$ ,  $Pb^{+2}$ .<sup>4</sup> The pH optimum of HRP is in the range of 6.0 to 6.5; activity at 7.5 is 84% of the maximum. The enzyme is most stable in the pH range of 5.0 to 9.0.<sup>5</sup>

Horseradish peroxidase is widely used as a 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, alkaline phosphatase, and B-galactosidase) and its glycosylation leads to lower non-specific binding.<sup>6</sup> Protocols describing the glutaraldehyde and periodate conjugation methodologies can be reviewed in Harlow, E. et al.<sup>7</sup>

#### Reagent

P 6140 is supplied as a crystalline suspension in 3.2 M  $(NH_4)_2SO_4$  solution containing potassium phosphate buffer, pH 6.0. All other peroxidases mentioned here are provided as essentially salt-free lyophilized powders. Additional differences are as follows: P6782 is similar to P8375 but packaged for convenience by mg solid. P8375 is packaged by units of activity. P2088 has been stabilized by chemical protection of the primary amines and maintains activity at low pH and higher temperature.<sup>8</sup> P8415 is a further purification of P8375.

#### Preparation Instructions

The choice of solvent will depend on the intended application. The powdered enzymes are soluble water or 0.1 M phosphate buffer, pH 6 (10 mg/ml). The suspension (P6140) may be diluted in water.

### Storage/Stability

The powdered peroxidases should be stored at 2 °C to 8 °C. If properly stored, these products have a shelf life of at least two years. Solutions lose <2 % of their activity per week if stored at – 20 °C or more than 10% per week if stored at room temp.

### Product Profile

RZ (Reinheitszahl): the absorbance ratio  $A_{403}/A_{275}$ . It is a measure of hemin content of the peroxidase, not enzyme activity. Even preparations with a high RZ value may have low enzymatic activity. For conjugating proteins such as antibodies to peroxidase, choose a peroxidase with an RZ value of at least 3.0

<u>Product</u>	<u>RZ value</u>
P6782	approx. 3.0
P8375	approx. 3.0
P2088	approx. 3.0
P8415	>3.0
P8125	approx. 1.0
P8250	approx. 2.0
P6140	approx. 3.0

Purpurogallin Unit Definition: One unit will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20 °C. This unit is equivalent to approx. 18 µM units per minute at 25 °C.

Purpurogallin Activity (units per mg solid):

P6782: 250-330

P8375: 250-330

P2088: 200-300

P8415: 250-330

P8125: approx. 80

P8250: 150-250

P6140: approx. 250 units per mg protein (Biuret)

ABTS Unit Definition: One unit will oxidize 1 µmole of 2, 2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) per minute at 25 °C, pH 5.0.

ABTS Activity (units per mg solid):

P6782: approx. 1000

### References

1. Delincee, H. and Radola, B.J., Eur. J. Biochemistry, **52**, 321-330 (1975).
2. Shannon, L.M., et al., J. Biol. Chem., **241**, 2166-2172 (1966).
3. Welinder, K.G., Eur. J. Biochem., **96**, 483-502 (1978).
4. Zollner, H., Handbook of Enzyme Inhibitors, 2<sup>nd</sup> 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).
7. Harlow, E. and Lane, D., Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, 346-348 (1988)
8. Ugarova, N.N., et al., Biochim. Biophys. Acta, **570**, 31-42 (1979).

**Tableted and Liquid Peroxidase Substrates for Immunoassays**

<b>Substrate</b>	<b>Products</b>	<b>Color Reaction</b>	<b>End Product</b>	<b>Applications</b>
2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) (ABTS)	A 3219 A 9941	Green	Soluble	ELISA
o-Phenylenediamine (OPD)	P 9187	Orange	Soluble	ELISA
3,3',5,5'-Tetramethyl-benzidine (TMB)	T 8665, T 3405	Blue	Soluble	ELISA
	T 0565	Deep Blue	Insoluble	Blotting
o-Dianisidine	D 9154	Yellow-Orange	Soluble	ELISA
5-Aminosalicylic Acid (5AS)	A 6178	Brown	Soluble	ELISA
3,3'-Diaminobenzidine (DAB)	D 7304 D 5905* D 4168* D 4293* D 4418* D 0426*	Brown     Blue-Black	Insoluble	Blotting
	*shown above D 7679, D 7304	Brown	Insoluble	Histochemistry
4-Chloro-1-Naphthol (4C1N)	C 6788	Blue	Insoluble	Blotting
3-Amino-9-Ethylcarbazole (AEC)	AEC-101,A 6926	Red	Insoluble	Blotting Histochemistry

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