**Product Information**

**Peroxidase from horseradish**  
Sigma Type VI  
Catalog Number **P8375**  
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$_2$O$_2$), and the resultant [HRP-H$_2$O$_2$] complex can oxidize a wide variety of hydrogen donors.

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

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.

Total molecular mass: ~44 kDa  
- Polypeptide chain: 33,890 Da  
- Hemin plus Ca$^{2+}$: ~700 Da  
- Carbohydrate: 9,400 Da

Extinction coefficient: $E_{\text{mM}}^{100} = 100$ (403 nm)

Optimal pH range: 6.0–6.5  
(ductivity 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: isozymes range from 3.0–9.0  
(at least seven isozymes)

Inhibitors: 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.

A review of glutaraldehyde and periodate conjugation methods has been published.

Peroxidase is also utilized for the determination of glucose and peroxides in solution.

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

Specific Activity: $\geq$250 units/mg solid (pyrogallol as substrate)

Unit definition (purpurogallin): One unit will form 1 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: 2.5–4.0  
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 4 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.

**Related Products**

Table 1.
Other Grades of HRP available

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>RZ value</th>
<th>Specific Activity (*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P6782</td>
<td>−3.0</td>
<td>250-330 units/mg solid</td>
</tr>
<tr>
<td>P2088</td>
<td>−3.0</td>
<td>200-300 units/mg solid</td>
</tr>
<tr>
<td>P8415</td>
<td>≥3.0</td>
<td>≥250 units/mg solid</td>
</tr>
<tr>
<td>P8125</td>
<td>−1.0</td>
<td>50-150 units/mg solid</td>
</tr>
<tr>
<td>P8250</td>
<td>≥1.8</td>
<td>150-250 units/mg solid</td>
</tr>
<tr>
<td>P6140</td>
<td>−3.0</td>
<td>≥225 units/mg protein</td>
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</tbody>
</table>

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

**References**

RBG,GCY,MAM 06/16-1
<table>
<thead>
<tr>
<th>Substrate</th>
<th>Catalog Number</th>
<th>Color Reaction</th>
<th>End Product</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,2’-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) (ABTS)</td>
<td>A3219, A9941</td>
<td>Green</td>
<td>Soluble</td>
<td>ELISA</td>
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<tr>
<td>o-Phenylenediamine (OPD)</td>
<td>P9187</td>
<td>Orange</td>
<td>Soluble</td>
<td>ELISA</td>
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<td>3,3’,5,5’-Tetramethylbenzidine (TMB)</td>
<td>T8665, T3405</td>
<td>Blue</td>
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<td>ELISA</td>
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<td>T0565</td>
<td>Deep Blue</td>
<td>Insoluble</td>
<td>Blotting</td>
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<tr>
<td>o-Dianisdine</td>
<td>D9154</td>
<td>Yellow-Orange</td>
<td>Soluble</td>
<td>ELISA</td>
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<tr>
<td>5-Aminosalicylic Acid (5AS)</td>
<td>A6178</td>
<td>Brown</td>
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<td>ELISA</td>
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<td>D7304, D5905, D4168, D4293, D4418, D7679</td>
<td>Brown</td>
<td>Insoluble</td>
<td>Blotting Histochemistry</td>
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<td></td>
<td>D0426</td>
<td>Blue-Black</td>
<td>Insoluble</td>
<td>Blotting</td>
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<tr>
<td>4-Chloro-1-Naphthol (4C1N)</td>
<td>C6788</td>
<td>Blue</td>
<td>Insoluble</td>
<td>Blotting</td>
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<td>3-Amino-9-Ethylcarbazole (AEC)</td>
<td>AEC101, A6926</td>
<td>Red</td>
<td>Insoluble</td>
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<td>CPS-1</td>
<td>CPS160, CPS1120, CPS1300</td>
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<tr>
<td>CPS-3</td>
<td>CPS350, CPS3100, CPS3500</td>
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<td>ELISA</td>
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<td>CPS-2</td>
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