Horse Radish Peroxidase Enzyme (HRP)

**Cat#** HRP16-N-500  
**Form**: powder  
**Storage**: dessicate at -20°C  
**Size**: 500 mg

**Description**

Horseradish peroxidase (HRP) is isolated from horseradish roots (Amoracia rusticana) and belongs to the ferroprotoporphyrin 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, mannone, mannosamine, and galactosamine, depending upon the specific isozyme. Its molecular weight (approx. 44 kDa) includes the polypeptide chain (33,890 Daltons), hemin plus Ca²⁺ (approx. 700 Daltons), and carbo-hydrate (9400 Daltons). At least seven isozymes of HRP exist. The isoelectric point for horseradish Peroxidase isozymes ranges from 3.0 - 9.0. HRP readily combines with hydrogen peroxide (H₂O₂) and the resultant [HRP-H₂O₂] complex can oxidize a wide variety of chromogenic hydrogen donors. 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, paminobenzoic acid, Cd⁺², Co⁺², Cu⁺², Fe⁺³, Mn⁺², Ni⁺², Pb⁺².

**Unit Definition**

The amount of enzyme that decomposes one micromole of hydrogen peroxide per minute at pH 7 and 25°C.

**Activity (units per mg solid):** >200 (Av. 200-300). Lot specific activity is provided on the vial.

**Solubility:** Soluble in distilled water or dilute buffer

**Assay Methods**

The rate of decomposition of hydrogen peroxide catalyzed by peroxidase, with 4-aminooantipyrine as hydrogen donor, is determined by measuring the increase in absorbance at 510 nm.

**Reagents**

1. 0.2 M Potassium phosphate, pH 7.0.
2. 0.0017 M Hydrogen peroxide. Prepare by diluting 1 ml of 30% H₂O₂ to 100 ml with distilled water. Further dilute 1 ml of this solution to 50 ml using 0.2 M phosphate buffer, pH 7.0. Prepare fresh daily.
3. 0.0025 M 4-Aminoantipyrine with 0.17 M phenol. Dissolve 810 mg phenol in 40 ml distilled water. Add 25 mg 4-aminooantipyrine and made the volume up to 50 ml with distilled water.
4. Enzyme (peroxidase) solution. Dissolve 1 mg/ml in distilled water. Just prior to use, dilute further with distilled water to yield a concentration of 0.05-0.25 U/ml

**Procedure**

1. Set spectrophotometer (equipped with strip chart recorder and temperature control) at 510 nm and 25°C.
2. Into the cuvette, pipette the following: Phenol/4-aminooantipyrine solution 1.4 ml  
   Hydrogen peroxide solution 1.5 ml
3. Mix the cuvette contents and incubate in spectrophotometer at 25°C for 3-4 minutes to achieve temperature equilibrium.
4. Establish blank rate, if any, at 510 nm.
5. Into the cuvette, pipette 0.1 ml enzyme solution (0.05-0.25 U/ml). Mix and record the increase in absorbance at 510 nm for 4-5 minutes.
6. Calculate µE₅₁₀ nm/min

**References**


For in vitro research use only

**Related Material available for ADI**

HRP with RZ values >2, >3
Antibodies to HRP and antibody conjugates
ELISA kits
Single solution, ready to use, TMB substrates for Blotting & ELISA
Chemiluminescence Substrates and Western blot kits

**HRP16-N-500 80410A**