ALPHA DIAGNOSTIC INTERNATIONAL

Product Data Sheet

Horse Radish Peroxidase Enzyme (HRP)

Cat# HRP16-N-500 Horse Radish Peroxidase (HRP) Enzyme (~200 u/mg) **Size:** 500 mg
Form: powder **Storage**: dessicate at -20oC

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, mannose, mannosamine, and galactosamine, depending upon the specific isozyme.2 Its molecular weight (approx. 44 kDa) includes the polypeptide chain (33,890 Daltons), hemin plus Ca2+ (approx. 700 Daltons), and carbo-hydrate (9400 Daltons).3 At least seven isozymes of HRP exist.2 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. 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. 4 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 9.0.5 Horseradish peroxidase is widely used as a label for immunoglobulins in many different immunochemistry leads to lower non-specific binding.6 Protocols describing the glutaraldehyde and periodate conjugation methodologies can be reviewed in Harlow, E. et al.

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).

Form and Storage

The powdered peroxidases should be stored in the freezer (–20°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.

Purity/Specificity

RZ (Reinheitszahl): the absorbance ratio A403/A275. 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. This product has RZ=1-2.

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 acivity 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-aminoantipyrine 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% H_2O_2 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-aminoantipyrine 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-aminoantipyrine 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

Delincee, H. and Radola, B.J., Eur. J. Biochemistry, 52, 321-330 (1975); Shannon, L.M., et al., J. Biol. Chem., 241, 2166-2172 (1966); Welinder, K.G., Eur. J. Biochem., 96, 483-502 (1978); Zollner, H., Handbook of Enzyme Inhibitors, 2nd Ed., Part A: 367-368 (1993); Schomberg, D., Salzmann, M., and Stephan, D., Enzyme Handbook 7, EC 1.11.1.7:1-6 (1993); Deshpande, S.S., Enzyme Immunoassays, From Concept to Product Development, Chapman and Hall, 169-171 (1996);

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
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