



Product Data Sheet

Horse Raddish Peroxidase Enzyme (HRP)

Cat# HRP15-N

Size: 1 mg
Storage: Store at 4°C

Form: powder

Description

Horseradish peroxidase (HRP) is isolated from horseradish roots (*Amoracia 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.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 (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, Cd²⁺, Co²⁺, Cu²⁺, Fe³⁺, Mn²⁺, Ni²⁺, Pb²⁺.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=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): 250-330

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): 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, 2nd 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).

For in vitro research use only

Related Material available for ADI

Single solution, ready to use, TMB substrates for Blotting & ELISA

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Chemiluminescence Substrates and Western blot kits (save by complete kit that includes Anti-rabbit IgG-HRP conjugate, blocking buffer, wash buffer, and Chemiluminescence Substrates for processing 15-30 std size blots).

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