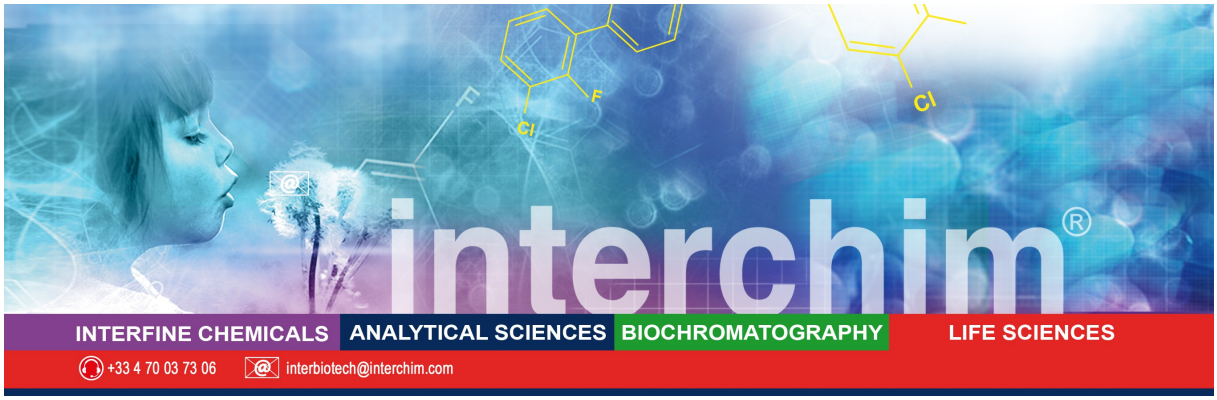
FT-146500



Horseradish Peroxidase

Peroxidase from Horseradish (HRP) is isolated from horseradish roots catalyzing the oxidation by hydrogen peroxide of a number of substrates, recommended for labeling probes used in immunoassays.

Reference	*Description*
UP146500, 250mg **UP146501, 1g**	**Peroxidase (horseradish), Rz >3** A chromatographically prepared, salt free, freeze-dried powder with an activity > 250 u/mg material and RZ > 3.0. This material typically has an 'Isoenzyme C' content of +/- 70%.
UP189161, 1g	**Peroxidase (horseradish), Rz >3, Isoform C** A chromatographically prepared, salt free, freeze-dried powder with an activity >250 u/mg material and RZ > 3.0. This material has an 'Isoenzyme C' content of approx. 90% [1]

Form: brown freeze-dried powder.
Dissolves readily at 5mg/ml In 0.1 M potassium phosphate pH 6.0 to give a clear brown solution.

Typical activity: 250-330 U/mg material
(1 unit = amount of enzyme that will catalyse the production of 1 mg of purpurogallin from pyrogallol in 20 seconds at +20°C and pH 6.0)

Typical Spectrophotometric data : RZ (Abs403nm/Abs275nm) 3.43 ; E 280 nm (1%) = 6.56

Storage conditions : desiccated at –15°C or below. (J)

Guide lines for use

Storage/Stability

Store desiccated at -15°C or below. Allow to come to room temperature before opening. Before returning to storage, re-dessicate under vacuum over silica gel for a minimum of four hours. Re-seal before returning to -15°C or below.

Handling

Allow to reach at room temperature before opening. Before returning to storage, redesiccate under vacuum over silica gel for a minimum of four hours.

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

The suspension should be stored at 2-8 °C. If properly stored, these products have a shelf life of at least two years.

Technical information

- **Peroxidase enzyme**

The **peroxidase** (Hydrogen-peroxide oxidoreductase; EC1.11.1.7) is a glycoprotein of 40 000-44 000Da. **Peroxidase from Horseradish** (HRP) is isolated from horseradish roots catalyzing the oxidation by hydrogen peroxide of a number of substrates such as ascorbate, ferrocyanide, cytochrome c and the leuco form of many dyes.
HRP has a molecular weight of approx. 44KDa. It includes the polypeptide chain (33,890 Daltons), hemin plus Ca2+ (approx. 700 Daltons), and carbohydrate (9400 Daltons) [r]. The carbohydrate composition consists of galactose, arabinose, xylose, fucose, mannose, mannosamine, and galactosamine, depending upon the specific isozyme [r].
Extinction coefficient is 100 when measured at 403 nm [r].
Isoelectric point ranges from 3.0 – 9.0 depending on isoenzymes.

- **Applications**

HRP is used notably:
-in a wide range of liquid clinical chemistry reagents, dry powder reagents and test strips.
-as a label in immunoassays such as ELISA, and in immunoblotting and histochemistry.
For conjugating proteins such as antibodies, it is recommended to use a highly active peroxidase, with an RZ value of at least 3.0.

HRP #UP189160 and UP189160 are recommended to label probes for immunoassays. HRP is the most desired label for antibodies, antigens, streptavidin or other probes, since it is the smallest and most stable of the three most popular enzyme labels (HRP, alkaline phosphatase, and β–galactosidase) and its glycosylation leads to lower non–specific binding [r]. It is also a faster reaction rate, i.e. than AP. Once conjugated to antibodies, its rapid turnover rate allows short incubation times and high sensitivity, especially with chemiluminescent substrates.

HRP can be conjugated to proteins by several different methods including glutaraldehyde, periodate oxidation, through disulfide bonds, and also via amino and thiol directed cross–linkers. See related products. Protocols describing the glutaraldehyde and periodate conjugation methodologies can be reviewed in Harlow, E. et al [r].

Peroxidase conjugates may give higher backgrounds than other enzyme conjugates, hence blocking steps may be necessary. See related products.

- **Activity**

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.
-chromogenic substrates suche as OPD, DAB, 4-CN, TMB,...
-chemiluminescent substrates such as luminol and isoluminol
-fluorogenic substrates such as tyramine, homovanillic acid, 4–hydroxyphenyl acetic acid.
See related products for detailed information on these substrates.

HRP activity is optimum at pH 7.0.

- **Activity - Unit Definition**

One **pyrogallol unit** is amount of enzyme that will form 1.0 mg purpurogallin from pyrogallol in 20 sec at pH 6.0 at 20°C. This purpurogallin (20 sec) unit is equivalent to approx. 18 µM units per min at 25°C.

Other used unit definitions are:

One **ABTS unit** will ABTS unit will oxidize 1µmole of ABTS per minute at 25°C at pH 5.0. Using ABTS as the substrate approx. four times the activity is observed over the pyrogallol system.

One **aminoantipyrine Unit** decomposes 1µmole of H2O2 per minute at 25°C, pH 7.0 using aminoantipyrine and phenol.
• 1 aminoantipyrine unit = 4.6 o-dianisidine units previously used by Worthington
• 1 aminoantipyrine unit = 0.62 ABTS units (µmole of dye oxidized per minute, pH 6.0, 25°C, 1.7 mM dye)
• 1 aminoantipyrine unit = 2 ABST units (µmole of dye oxidized per minute, pH 5.0, 25°C, 8.7 mM dye)
• 1 aminoantipyrine unit = 0.5 guiacol units (µmole of guiacol oxidized per minute, pH 7.0, 25°C)
• 1 aminoantipyrine unit = 0.5 pyrogallol to purpogallin unit (mg of product per 20 seconds, pH 6.0, 20°C)
• 1 aminoantipyrine unit = 5 pyrogallol to purpogallin units (µmole of product per minute at pH 6.0, 30°C)

- **pH Dependence**

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

- **Grade - RZ**

The RZ (Reinheitzahl) factor is the absorbance ratio, Abs403nm/Abs275nm.
RZt has been used as an indication of purity. However, it measures essentially hemin content and is not necessarily indicative of enzymatic activity: Shannon et al., JBC, 241, 266 (1966) reports that this ratio for the isozymes varies from 2.50 to 4.19. There is also an influence exerted by buffer and pH.

- **Substrates**

Interchim offers several chromogenic, fluorescent and chemiluminescent substrates, inlcuding formulations optimized for blotting, histology and ELISA applications.

- **Inhibitors**

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}.4

Related products

HRP substrates: Hydrogen Peroxide #15983Q

- chromogenic

ABTS 'powder #UP423876, tablets #UP423879, solution #732550
DAB; op-Metal Enhanced DAB kit for IHC #679921, tablets #UP732310, powder #UP01012G
TMB: powder #UP15426D, solution for ELISA #UP664780
AEC: powder #UP036310
4-CN: powder #UP007790
OPD: tablets 270861

- fluorigenic

ADHP: powder #FP-39423A, assay kit #HS624A
Luminol: powder #04242F
Resorufin: powder #95432A

- luminogenic

UptiLight HRP Chemiluminescent Substrates (ECL):
.for blotting (one component in spray #BM4961, (one component, dopper #BM4963), (femto range detection #58372A)
.for ELISA (pico range detection #36349A (Femto range detection #996201)

HRP conjugated probes: secondary antibodies, primary antibodies, (strep)avidins,...

Buffers and Saturating agents:
PBS buffer (Phosphate Buffered Saline): #UP68723A
TBS buffer (Tris Buffer Saline) #UP74004A; with non fat milk #GS4160 or TBS with Tween20 #UPGS4200
SeaBlock agent #UP40301A
BSA Biotech grade #UPQ84170 (powder) or #UP900130 (solution 30%)
BioBlock Saturating agent for (W, N, S) blotting (in TBS) #N13650
Non fat milk powder #768701
Gelatin, APO-Peroxidase blocker (for most demanding applicaitions).

Other peroxidases : grades with Rz >1 (#857280), mainly acidic isoform (#882990), for clinical biochemistry, type I to IX, for other source that horseradish... Please inquire.

For any question, please contact :
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