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PRODUCT INFORMATION
Lignin-Peroxidase from ***Phanerochaete chrysosporium***

Diarylpropane:oxygen, hydrogen-peroxide oxidoreductase

EC 1.11.1.14
CAS 93792-13-3

Source: ***Phanerochaete chrysosporium***

Reaction: **3,4-dimethoxybenzyl alcohol + H_2O_2 ⟶**

3,4-dimethoxybenzaldehyde + 2 H_2O

Activity: **The specific activity depends on the production charge, please ask us for the current data.**

Unit definition: **One unit will oxidize 1.0 µmole of 3,4-dimethoxybenzyl alcohol (= veratryl alcohol) per minute at pH 3.3 at 25°C**

Assay method: **The reaction velocity of the enzyme-catalyzed reaction is determined by the increase in absorbance at 310 nm.**

Form: **Freeze-dried powder**

Appearance: **Brownish powder**

Stability: **Stable up to 12 months, when stored at -18°C**

pH-Optimum: **3,4-dimethoxybenzyl alcohol oxidation (100 mM sodium tartrate buffer): pH 3.3**

Notes: **A hemeprotein, involved in the oxidative breakdown of lignin by white-rot basidiomycete fungi. The enzyme from P. chrysosporium oxidizes typical peroxidase dye substrates at the heme iron, a reaction involving the formation of compound II (FeIV=O); it also oxidizes 3,4-dimethoxybenzylalcohol (veratryl alcohol) to the radical cation. The bound veratryl alcohol radical is proposed to bring about the oxidative cleavage of C-C and ether (C-O-C) bonds in lignin model compounds of the diarylpropane and arylpropane-aryl ether type.**

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Enzymatic Assay of Lignin-Peroxidase

Diarylpropane:oxygen,hydrogen-peroxide oxidoreductase EC 1.11.1.14

Principle: Lignin peroxidase catalyzes the oxidation of non-phenolic aromatic rings into aryl cation radicals by H_2O_2. Aryl cation radicals are unstable and undergo various following reactions. A typical example is the oxidation of veratryl alcohol into veratryl aldehyde via the intermediary formation of veratryl cation and benzyl radicals [1].

Reaction: 3,4-dimethoxybenzyl alcohol + H_2O_2 ⟶ 3,4-dimethoxybenzaldehyde + 2 H_2O

Method: Continuous spectrophotometric determination of the increase in absorbance at 310 nm resulting from the formation of veratryl aldehyde (ε_{310nm} = 9,300 M^{-1} cm^{-1}) [2].

Conditions:

Temperature:	25°C
pH:	3.3
Wavelength:	310 nm
Light path:	1 cm

Unit definition: The amount of enzyme that oxidizes 1.0 µmole of 3,4-dimethoxybenzyl alcohol (veratryl alcohol) per minute at pH 3.3 at 25°C.

Reagents:

Sodium-tartrate buffer	100 mM, pH 3.3
H_2O_2	20 mM
Veratryl alcohol	50 mM
Enzyme solution	ca. 0.5 U/ml

All reagents should be prepared from deionized, distilled water. Dissolve the enzyme at a concentration of 0.4 mg/ml in distilled water. Dilute further in distilled water if necessary.

Procedure: *Pipette into a suitable quartz cuvette:*

buffer	675 µl
veratryl alcohol	75 µl
Enzyme solution	200 µl

Incubate at 25°C and check the temperature, place the reaction mixture into the photometer and monitor the absorbance until constant, start the enzymatic reaction by adding

H_2O_2	50 µl

<u>Immediately</u> mix and record the increase in absorbance at 270 nm for ca. 1 minute. Calculate the ΔA/min from the linear range of the curve (initial range: standardized as 10 sec after start).

Calculation:

$$\text{Volume activity} = \frac{\Delta A/\text{min} \cdot 1000 \cdot \text{volume}_{\text{reaction mixture (µl)}}}{9{,}300 \cdot \text{volume}_{\text{enzyme solution (µl)}}}$$

$$= \text{[U / ml enzyme solution]}$$

References:

[1] Schoemaker H. et al. (1994). FEMS Microbiol. Rev. 13: 321-332.

[2] Tien, M., Kirk, T.K. (1984). Proc. Nat. Acad. Sci. U.S.A. 81: 2280-2284.