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PRODUCT INFORMATION
Versatile Peroxidase from ***Bjerkandera adusta***

Mn(II): hydrogen-peroxide oxidoreductase EC 1.11.1.16, CAS 42613-30-9, 114995-15-2

Source: ***Bjerkandera adusta***

Reaction: **2 Mn(II) + 2 H^+ + H_2O_2 → 2 Mn(III) + 2 H_2O**

3,4-dimethoxybenzyl alcohol + H_2O_2 → 3,4-dimethoxybenzyl aldehyde + 2 H_2O

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

Unit definition: **The amount of one unit will oxidize 1.0 µmole of Mn(II) per minute at pH 4.5 at 25°C, or 0.05 µmole of 3,4-dimethoxybenzyl alcohol per minute at pH 3.0 at 25°C.**

Assay method: **The reaction velocity of the enzyme-catalyzed reaction is determined by the increase in absorbance at 270 nm resulting from the formation of Mn(III)-malonate, or in absorbance at 310 nm resulting from the formation of 3,4-dimethoxybenzyl aldehyde.**

Form: **Freeze-dried powder**

Appearance: **Brownish powder**

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

pH-Optimum: **Mn(II) oxidation (50 mM malonate buffer): pH 4.5; 3,4-dimethoxybenzyl alcohol (veratryl alcohol) (100 mM tartrate buffer): pH 3.0**

Notes: **Versatile peroxidase (syn. manganese-lignin peroxidase) is a new ligninolytic enzyme, combining catalytic properties of manganese peroxidase (oxidation of Mn(II)), lignin peroxidase (Mn-independent oxidation of non-phenolic aromatic compounds) and plant peroxidase (oxidation of hydroquinones and substituted phenols) [1].**

References: **[1] Ruiz-Duenas, F.J. et al. (2001). Biochem. Soc. Trans 29 (2): 116-122.**

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Enzymatic Assay of Versatile Peroxidase

Versatile Peroxidase / Mn(II)

Principle: Versatile peroxidase catalyzes the oxidation of Mn(II) to Mn(III) by H2O2. The highly reactive Mn(III) is stabilized via chelation in the presence of dicarboxylic acid.[1]

Reaction: $2\ Mn(II) + 2\ H^+ + H_2O_2 \longrightarrow 2\ Mn(III) + 2\ H_2O$

Method: Continuous spectrophotometric determination of the increase in absorbance at 270 nm resulting from the formation of Mn(III)-malonate (ε_{270nm} = 11,590 $M^{-1}\ cm^{-1}$) [2].

Conditions:

Temperature:	25°C
pH:	4.5
Wavelength:	270 nm
Light path:	1 cm

Unit definition: The amount of enzyme that oxidizes 1.0 µmole of Mn(II) per minute at pH 4.5 and 25°C.

Reagents:

Sodium-malonate buffer	50 mM, pH 4.5
H_2O_2	20 mM
$MnCl_2$	20 mM
Enzyme solution	ca. 0.5 U/ml

All reagents should be prepared from deionized, distilled water. Dissolve the enzyme at a concentration of 0.3 mg/ml in distilled water. Dilute further in distilled water if necessary.

Procedure: *Pipette into a suitable quartz cuvette:*

buffer	690 µl
$MnCl_2$ -solution	100 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	10 µl

Immediately 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:

$$\text{Volume activity} = \frac{\Delta A/\text{min} \cdot 1000 \cdot \text{volume}_{\text{reaction mixture (µl)}}}{11{,}590 \cdot \text{volume}_{\text{enzyme solution (µl)}}}$$

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

References:

[1] Hofrichter, M. et al. (1998). Appl. Environ. Microbiol. 64: 399-404.

[2] Wariishi, H. et al. (1992). J. Biol. Chem. 2676: 23688-23695.