
PRODUCT INFORMATION

Manganese Peroxidase from *Nematoloma frowardii*

Mn(II): hydrogen-peroxide oxidoreductase

EC 1.11.1.13
CAS 114995-15-2

Source: ***Nematoloma frowardii***

Reaction: **$2 \text{ Mn(II)} + 2 \text{ H}^+ + \text{ H}_2\text{O}_2 \longrightarrow 2 \text{ Mn(III)} + 2 \text{ H}_2\text{O}$**

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

Unit definition: **One unit will oxidize 1.0 μ mole of Mn(II) per minute at pH 4.5 at 25°C.**

Assay method: **The reaction velocity of the enzyme-catalyzed reaction is determined by the increase in absorbance at 270 nm.**

Form: **Lyophilisate**

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**

Notes: **This hemoprotein is involved in the oxidative degradation of lignin in white-rot basidiomycetes.**

Enzymatic Assay of Manganese-Peroxidase

Mn(II): hydrogen-peroxide oxidoreductase EC 1.11.1.13

Principle: Manganese 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: $2 \text{Mn(II)} + 2 \text{H}^+ + \text{H}_2\text{O}_2 \longrightarrow 2 \text{Mn(III)} + 2 \text{H}_2\text{O}$

Method: Continuous spectrophotometric determination of the increase in absorbance at 270 nm resulting from the formation of Mn(III)-malonate ($\epsilon_{270\text{nm}} = 11,590 \text{ M}^{-1} \text{ cm}^{-1}$) [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 ₂ O 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 ₂ O ₂	10 μl
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Immediately mix and record the increase in absorbance at 270 nm for ca. 1 minute. Calculate the $\Delta A/\text{min}$ from the linear range of the curve (initial range: standardized as 10 sec after start).

Calculation:

$$\begin{aligned} \text{Volume activity} &= \frac{\Delta A/\text{min} \cdot 1000 \cdot \text{volume}_{\text{reaction mixture}} (\mu\text{l})}{11,590 \cdot \text{volume}_{\text{enzyme solution}} (\mu\text{l})} \\ &= [\text{U} / \text{ml enzyme solution}] \end{aligned}$$

References: [1] Hofrichter, M. et al. (1998). Appl. Environ. Microbiol. 64: 399-404.
[2] Wariishi, H. et al. (1992). J. Biol. Chem. 267: 23688-23695.