
PRODUCT INFORMATION

Laccase from *Cerrena unicolor*

Benzenediol:oxygen oxidoreductase

EC 1.10.3.2, CAS 80498-15-3

Source: *Cerrena unicolor*

Reaction: 4 benzenediol + O₂ → 4 benzosemiquinone + 2 H₂O

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 ABTS per minute at pH 4.5 and 25°C.

Assay method: The velocity of the enzyme-catalyzed reaction is determined by the increase in absorbance at 420 nm.

Form: Freeze-dried powder

Appearance: Brownish powder

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

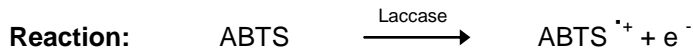
pH-Optimum: ABTS-oxidation (50 mM citrate/phosphate buffer): pH 4.5

Notes: A group of multi-copper proteins of low specificity acting on both o- and p-quinols, and often acting also on aminophenols and phenylenediamine. The semiquinone may react further either enzymically or non-enzymically.

Enzymatic Assay of Laccase

Laccase / ABTS

Principle: Fungal laccase oxidizes 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonate) (ABTS) to the stable green-colored cation radical (ABTS^{•+}).



Method: Continuous spectrophotometric determination of the increase in absorbance at 420 nm [1;2].

Conditions: Temperature: 25°C
pH: 4.5
Wavelength: 420 nm
Light path: 1 cm

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

Reagents: Phosphate-citrate-buffer 100 mM, pH 4.5
ABTS 6 mM
Enzyme solution ca. 0.1 units / ml

All reagents should be prepared from deionized, distilled water. Prepare an enzyme solution in deionized, distilled water containing approximately 0.1 units / ml.

Procedure: *Pipette into a suitable quartz cuvette:*

buffer	700 μl
Enzyme solution	100 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

ABTS-solution	200 μl
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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:

$$\begin{aligned} \text{Volume activity} &= \frac{\Delta A/\text{min} \cdot 1000 \cdot \text{volume}_{\text{reaction mixture}} (\mu\text{l})}{36,000 \cdot \text{volume}_{\text{enzyme solution}} (\mu\text{l})} \\ &= [\text{U} / \text{ml enzyme solution}] \end{aligned}$$

References: [1] Childs, R.E., Bardsley, W.G. (1975). Biochem. J. 145: 93-103.
[2] Wolfenden, B.S., Willson, R.L. (1982). J. Chem. Soc. Perkin. Trans. 11: 805-812.