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Analytik · Beratung · Biotechnologie

PRODUCT INFORMATION
Laccase from ***Cerrena unicolor***

Benzenediol:oxygen oxidoreductase EC 1.10.3.2, CAS 80498-15-3

Source: *Cerrena unicolor*

Reaction: **4 benzenediol + O_2 ⟶ 4 benzosemiquinone + 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 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 specifity 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.**

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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 ($ABTS^{\bullet+}$).

Reaction: $ABTS \xrightarrow{\text{Laccase}} ABTS^{\bullet+} + e^-$

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

<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:

$$\text{Volume activity} = \frac{\Delta A/\text{min} \cdot 1000 \cdot \text{volume}_{\text{reaction mixture (µl)}}}{36{,}000 \cdot \text{volume}_{\text{enzyme solution (µl)}}}$$

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

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.

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