漆酶

Specification

One unit corresponds to the amount of enzyme which converts 1 µmol catechole per minute at pH 6.0 and 25 °C.

Reagents

- Citric acid, stock solution, prepared from:
- 1 M sodium hydroxide solution
- 1,2-Dihydroxybenzene
- Laccase

Solvents/Sample

A)　50 mM sodium citrate, pH 6.0, 50 mL:

Add 2.5 mL 1 M citric acid stock solution (MW 192.43) to 40 mL H2O, adjust pH to 6.0 with 1 M NaOH and bring to 50 mL.

B)　Substrate, 25 mL:

Dissolve 275 mg 1,2-dihydroxybenzene in 25 mL buffer A.

C)　Enzyme sample, ~ 0.8 U/mL:

Dissolve the laccase in cold buffer A and dilute.

Method/Procedure:

Wavelength:	415 nm	Cuvettes:	1 mL polystyrene
Reaction volume:	1.050 mL	Temperature:	25 °C
Reaction time:	10 min	pH:	6.0

Pipet the following (mL) into cuvettes:

	Sample	Blank
Substrate B	1.0	1.0
Buffer A	—	0.05

Adjust temperature to 25 ° C. Start by addition of:		
Then add:		
Enzyme C	0.05	—
Mix and measure for 10 minutes at 25 ℃		

Calculation：

The maximum linear rate (Abs/min) is entered into the calculation:

$$\text{Volumetric Activity (U/mL)} = \frac{\text{Abs/min} \times V_{tot} \times F}{1 \times d \times v_{Pr}}$$

$$\text{Specific Activity (U/mg)} = \frac{\text{volumetric activity}}{\text{konz}_{Pr}}$$

Abs/min =Abs/min (sample) – Abs/min (blank)

V_{tot}：total volume of the assay (mL)

F：dilution factor

1：absorption coefficient (1 cm^2/μmol at 415 nm)

D：path length of the cuvette (1 cm)

v_{Pr}：volume of the enzyme sample (mL)

$konz_{Pr}$：concentration of the enzyme sample added (mg/mL)