ProductInformation

TYROSINASE
from mushroom
Sigma Prod. No. T7755

CAS NUMBER: 9002-10-2
EC NUMBER: 1.14.18.1
SYNONYMS: Monophenol monooxygenase; Polyphenol oxidase; Catechol oxidase; Monophenol, dihydroxyphenylalanine: oxygen oxidoreductase

PHYSICAL DESCRIPTION:

Appearance: Gray-brown to dark brown powder
Isoelectric point (pI): 4.7-~5[3]
Kinetic Parameter: pH optimum is 6-7

STRUCTURE:

Molecular weight: 128,000∀5% by sedimentation velocity diffusion; 133,000∀10,000 by light-scattering measurements.[1] Reported as 119.5 kDa by electrophoresis.[2]

INHIBITORS:

"Tyrosinase is characterized by a peculiar irreversible inactivation reaction which occurs during the oxidation of o-diphenols to o-quinones", particularly catechol.[4] It is also inhibited by compounds that complex with copper, benzoic acid and cyanide. The benzoic acid inhibition is competitive with catechol; the cyanide inhibition is competitive with oxygen, noncompetitive with catechol.[5]

SUBSTRATES AND SPECIFICITY:

Tyrosinase is a copper-containing oxidase which has activity for both catechols and cresol. It is responsible for browning reactions. The enzyme is reported to have two binding sites for aromatic substrates and a different binding site for oxygen- the copper site.[5] The copper is probably Cu(I), with inactivation involving oxidation to Cu(II) ion.[6]

This enzyme is assayed for tyrosinase activity using L-tyrosine as substrate, for polyphenol oxidase using L-dihydroxyphenylalanine, and for catechol oxidase activity using catechol and L-ascorbic acid. (These assays are available on request from Research Technical Service.) Another assay method of interest is based on the stoichiometric reaction of cysteine with orthoquinones produced during the enzymatic oxidation of phenols.[8] A continuous spectrophotometric method of determining diphenolase activity has been proposed using 3,4-dihydroxymandelic acid.[9]

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METHOD OF PREPARATION:

Tyrosinase, T7755, is isolated from the mushroom species Agaricus bisporus.[1,7] The product was dialyzed against water prior to lyophilization.

STORAGE / STABILITY AS SUPPLIED:

If the product is stored at 37EC, 40-60% activity is lost in 7 days; if stored at -20EC, less than 5% activity is lost per year.[6]

SOLUBILITY / SOLUTION STABILITY:

Tyrosinase dissolves at 2 mg/mL in 50 mM potassium phosphate buffer, pH 6.5, to give a clear brown solution.

The enzyme is rapidly denatured by a 1% SDS solution.[2] It dissociates into subunits in saturated urea and is completely inactivated. The dissociation is reversible, but the inactivation is not.[1] Solutions retain activity for several days at 2EC and for several weeks frozen at -20EC.[1]

UNIT DEFINITION:

One unit will cause an increase in A280 of 0.001 per minute at pH 6.5 at 25EC in a 3 mL reaction mix containing L-tyrosine.

REFERENCES:

1. Kertez, D. and Zito, R., *Biochim. Biophys. Acta*, 96, 447 (1965).
2. Gillespie, J.P. et al., *Comp. Biochem. Physiol.,* 98C, 351-358 (1991). (Sigma's T7755 was used as the control).
3. *Phytochemistry,* 20, 1481-1485 (1981).
4. Garcia-Canovas, F. et al., *Biochim. Biophys. Acta*, 912, 417-423 (1987).
5. Duckworth, H.W. and Colman, J.E., *J. Biol. Chem.*, 245, 1613-1625 (1970).
6. Kertez, D., *Biochem. Biophys. Res. Commun.,* 49, 1208 (1972)
7. Sigma Production.
8. Gauillard, F. et al., *Anal. Biochem.*, 215, 59-65 (1993).
9. Rodriguez-Lopez, J.N. et al., *Anal. Biochem.*, 195, 369-374 (1991).