Enzymatic Assay of CATECHOL OXIDASE
(EC 1.14.18.1)

PRINCIPLE:

Catechol + O_2 $\xrightarrow{\text{Catechol Oxidase}}$ O-Benzoquinone + H_2O

O-Benzoquinone + Ascorbic Acid ——> Catechol + Dehydro-Ascorbic Acid

CONDITIONS: T = 25°C, pH = 6.5, A_{265nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Potassium Phosphate Buffer, pH 6.5 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Prod. No. P-5379. Adjust to pH 6.5 at 25°C with 1 M NaOH.)

B. 5 mM Catechol Solution
(Prepare 10 ml in Reagent A using Catechol, Prod. No. C-9510. **PREPARE FRESH.**)

C. 2.1 mM L-Ascorbic Acid Solution
(Prepare 10 ml in Reagent A using L-Ascorbic Acid, Sodium Salt, Prod. No. A-7631. **PREPARE FRESH.**)

D. 0.065 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 10 ml in Reagent A using Ethylenediaminetetraacetic Acid, Disodium Salt, Stock No. ED2SS.)

E. Catechol Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 500 - 1000 units/ml of Catechol Oxidase in cold Reagent A.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable quartz cuvettes:

	Test	Blank
Reagent A (Buffer)	2.60	2.80
Reagent B (Catechol)	0.10	0.10
Reagent C (Ascorbic Acid)	0.10	------[1]
Reagent D (EDTA)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the A_{265nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent E (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the decrease in A_{265nm} for approximately 5 minutes. Obtain the ΔA_{265nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{(\Delta A_{265nm}/\text{min Test} - \Delta A_{265nm}/\text{min Blank})}{(0.001)\ (\text{mg enzyme/RM})}$$

0.001 = The change in A_{265nm}/minute per unit of Catechol Oxidase at pH 6.5 at 25°C in a 3 ml reaction mix
RM = Reaction Mix (final volume = 3 ml)

UNIT DEFINITION:

One unit will cause the change in A_{265nm} of 0.001 per minute at pH 6.5 and 25°C in a 3 ml reaction mix containing catechol and L-ascorbic acid.

FINAL ASSAY CONCENTRATION:

In a 3 ml reaction mix, the final concentrations are 50 mM potassium phosphate, 0.17 mM catechol, 0.07 mM L-ascorbic acid and 50 - 100 units of catechol oxidase.

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REFERENCES:

Dawson, C.R., and Magee, R.J. (1955) *Methods in Enzymology* **II**, 817-821.

Marumo, K., and Waite, J.H. (1986) *Biochim. Biophys. Acta* **872**, 98-103.

NOTES:

1. Ascorbic acid is not included in the blank to produce a true blank. Possible interaction with catechol and ascorbic acid might give false readings.

2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.