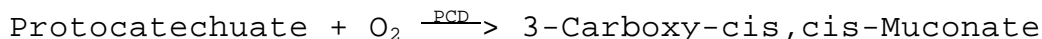


**Enzymatic Assay of PROTOCATECHUATE 3,4-DIOXYGENASE
(EC 1.13.11.3)**

PRINCIPLE:



Abbreviation used:

PCD = Protocatechuate 3,4-Dioxygenase

CONDITIONS: T = 37°C, pH = 7.5, A_{290nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 200 mM Acetic Acid Solution (Acet Acid)
(Prepare 100 ml in deionized water using Acetic Acid, Glacial, Sigma Prod. No. A-6283.)
- B. 50 mM Tris Acetate Buffer, pH 7.5 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.5 at 25°C with Reagent A.)
- C. 0.40 mM Protocatechuic Acid Solution (PCA)
(Prepare 50 ml in Reagent B using Protocatechuic Acid, Sigma Prod. No. P-5630.)
- D. Protocatechuate 3,4-Dioxygenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.15 mg/ml of Protocatechuate 3,4-Dioxygenase in ice cold Reagent B.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent C (PCA)	3.00	3.00

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PROCEDURE: (continued)

Equilibrate to 37°C and monitor the $A_{290\text{nm}}$ until constant using a suitably thermostatted spectrophotometer. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent D (Enzyme Soln)		0.10
Reagent B (Buffer)	-----	----- 0.10

Immediately mix by inversion and record the decrease in $A_{290\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{290\text{nm}}$ /minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{290\text{nm}}/\text{min Test} - r A_{290\text{nm}}/\text{min Blank})(3.1)(\text{df})}{(3.8)(0.1)}$$

3.1 = Total volume (in milliliters) of assay

df = Dilution factor

3.8 = Millimolar extinction coefficient¹ of protocatechuic acid at 290 nm

0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will oxidize 1.0 μmole of protocatechuate to 3-carboxy-cis,cis-muconate per minute at pH 7.5 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.10 ml reaction mix, the final concentrations are 50 mM Tris, 0.39 mM protocatechuic acid, and 0.015 mg protocatechuate 3,4-dioxygenase.

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REFERENCES:

MacDonald, D.L., Stanier, R.Y. and Ingraham, J.L. (1954) *Journal of Biological Chemistry* **210**, 809-820

Fujisawa, H. and Hayaishi, O. (1968) *Journal of Biological Chemistry* **243**, 2673-2681

NOTES:

1. The millimolar extinction coefficient of protocatechuic acid is described in MacDonald, D.L. et al. (1954).
2. This assay is based on the cited references.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.
4. Use 0.10 ml aliquots of Reagent D only. Do not vary the concentration of the enzyme in the Reaction Mix.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.