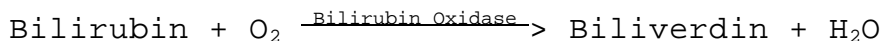


**Enzymatic Assay of BILIRUBIN OXIDASE
(EC 1.3.3.5)**

PRINCIPLE:



CONDITIONS: T = 37°C, pH = 8.4, A_{440nm}, Light path 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 200 mM Tris HCl Buffer, pH 8.4 at 37°C
(Prepare 100 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253. Adjust to pH 8.4 at 37°C with 1 M NaOH.)
- B. 0.002% (w/v) Bilirubin Solution (Bilirubin)
(Prepare 50 ml by initially suspending 1 mg of Bilirubin, Mixed Isomers, Sigma Prod. No. B-4126, in 1 ml of deionized water. Dissolve it by adding one drop of 2 N NaOH and then bring to a final volume of 50 ml with Reagent A.)
- C. Bilirubin Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.025 - 0.050 unit/ml of Bilirubin Oxidase in cold deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Bilirubin)	2.90	2.90

Equilibrate to 37°C. Then add:

Reagent C (Enzyme Solution)	0.10	-----
Deionized Water	-----	0.10

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PROCEDURE: (continued)

Immediately mix by inversion and record the decrease in $A_{440\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{440\text{nm}}$ /minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{440\text{nm}}/\text{min Test} - r A_{440\text{nm}}/\text{min Blank})(3)(\text{df})}{(56.3)(0.10)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

56.3 = Millimolar extinction coefficient of Bilirubin
at 440nm^1

0.10 = 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 bilirubin per minute at pH 8.4 at 37°C .

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 193 mM Tris, 0.002% (w/v) bilirubin, and 0.0025 - 0.0050 unit bilirubin oxidase.

REFERENCE:

Murao, S. and Tanaka, N. (1981) *Agric. Biol. Chem.* **45**,
2383-2384

NOTES:

1. The extinction coefficient was determined by the supplier of the enzyme to Sigma.
2. This assay is based on the cited reference.

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NOTES: (continued)

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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