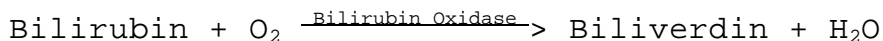


**Enzymatic Assay of BILIRUBIN OXIDASE  
(EC 1.3.3.5)**

**PRINCIPLE:**



**CONDITIONS:** T = 37°C, pH = 8.4, A<sub>440nm</sub>, 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  $440\text{nm}^1$

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  $\mu\text{mole}$  of bilirubin per minute at pH 8.4 at  $37^\circ\text{C}$ .

**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.**