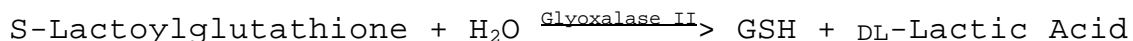


**Enzymatic Assay of GLYOXALASE II
(EC 3.1.2.6)**

PRINCIPLE:



Abbreviations:

GSH = Reduced Glutathione

CONDITIONS: T = 25°C, pH = 7.4, A_{240nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Tris HCl Buffer, pH 7.4 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.4 at 25°C using 1 M HCl.)
- B. 0.76% (w/v) S-Lactoylglutathione Solution (SLG)
(Prepare 10 ml in deionized water using S-Lactoylglutathione, Free Acid, Sigma Prod. No. L-7140.)
- C. Glyoxalase II Enzyme Solution (Glyox II)
(Immediately before use, prepare a solution containing 0.2 - 0.4 unit/ml of Glyoxalase II in cold deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.90	2.90
Reagent B (SLG)	0.07	0.07

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PROCEDURE:

Mix by inversion and equilibrate to 25°C. The A_{240nm} should be 0.6 - 0.8. If not, add more Reagent B so that the A_{240nm} is within this range. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent C (Enzyme Solution)	0.10	0.10
Deionized Water	-----	0.10

Immediately mix by inversion and record the decrease in A_{240nm} for approximately 3 minutes. Obtain the ΔA_{240nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{240\text{nm}}/\text{min Test} - \Delta A_{240\text{nm}} \text{ min Blank})(3.07)(\text{df})}{(3.37)(0.1)}$$

3.07 = Total volume (in milliliters) of assay

df = Dilution factor

3.37 = Millimolar extinction coefficient of
S-Lactoylglutathione at 240 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 hydrolyze 1.0 μmole of S-lactoylglutathione per minute at pH 7.4 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.07 ml reaction mix, the final concentrations are 48 mM Tris, 0.0176% (w/v) D-lactoylglutathione and 0.02 - 0.04 unit glyoxalase II.

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REFERENCES :

Racker, E. (1951) *Journal of Biological Chemistry* **190**, 685

NOTES :

1. This assay is based on the cited reference.
2. 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.