

**Enzymatic Assay of GLYOXALASE I
(EC 4.4.1.5)**

PRINCIPLE:

Methylglyoxal + GSH $\xrightarrow{\text{Glyoxalase I}}$ S-Lactoylglutathione

Abbreviations:

GSH = Reduced Glutathione

CONDITIONS: T = 25°C, pH 6.6, A_{240nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Potassium Phosphate Buffer, pH 6.6 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.6 with 1 M KOH.)
- B. 0.2% (v/v) Methylglyoxal Solution (Meth-Glyox)
(Prepare 50 ml in deionized water using Methylglyoxal, Sigma Prod. No. M-0252.¹)
- C. 2.0% (w/v) Reduced Glutathione Solution, pH 6.6 at 25°C (GSH)
(Prepare 10 ml in deionized water using Glutathione, Reduced Form, Free Acid, Sigma Prod. No. G-4251. Adjust to pH 6.6 at 25°C with solid Sodium Bicarbonate, Sigma Prod. No. S-8875. **PREPARE FRESH.**)
- D. 10 mM Potassium Phosphate Buffer with 0.1% (w/v) Bovine Serum Albumin, pH 7.4 at 25°C (Enzyme Diluent)
(Prepare 10 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, and Albumin, Bovine, Sigma Prod. No. A-4503. Adjust to pH 7.4 at 25°C with 1 M KOH.)
- E. Glyoxalase I Enzyme Solution (Glyoxalase I)
(Immediately before use, prepare a solution containing 0.25 - 0.50 unit/ml of Glyoxalase I, Sigma Prod. No. G-4252, in Reagent D.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.35	2.35
Reagent B (Meth-Glyox)	0.50	0.50
Reagent C (GSH)	0.05	0.05

Mix by inversion and equilibrate to 25°C. Monitor the $A_{240\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent E (Glyoxalase I)	0.10	-----
Reagent D (Enzyme Diluent)	-----	0.10

Mix by inversion and record the increase in $A_{240\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{240\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{240\text{nm}}/\text{min Test} - r A_{240\text{nm}}/\text{min Blank})(3)(\text{df})}{(3.37)(0.1)}$$

3 = 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 form 1.0 μmole of S-lactoylglutathione from methylglyoxal and reduced glutathione per minute at pH 6.6 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 79 mM potassium phosphate, 0.033% (w/v) reduced glutathione, 0.003% (w/v) bovine serum albumin, and 0.025 - 0.05 unit of glyoxalase I.

REFERENCE:

Davis, K.A. and Williams, G.R. (1969) *Canadian Journal of Biochemistry* **47**, 553

NOTES:

1. Wear a respirator when handling methylglyoxal directly from the bottle.
2. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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