Determination of the Concentration and Molecular Weight of METHYLGLYOXAL

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: Spectrophotometric

REAGENTS:

A. 1 M Potassium Phosphate Buffer, pH 6.6 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379. Adjust to pH 6.6 with 5 M KOH.)

B. 0.92 mM Methylglyoxal Solution (Meth-Glyox)
(Immediately before use, prepare using deionized water.)[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, Prod. No. G-4251. Adjust to pH 6.6 at 25°C with solid Sodium Bicarbonate, Prod. No. S-8875.)

D. 10 mM Potassium Phosphate Buffer with 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, Prod. No. P-5379, and Albumin, Bovine, Prod. No. A-4503 or equivalent. 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 2.5 units/ml of Glyoxalase I, Prod. No. G-4252, in Reagent D.)

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PROCEDURE:

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

	Test	Blank
Deionized Water	2.25	2.75
Reagent A (Buffer)	0.10	0.10
Reagent B (Meth-Glyox)	0.50	------
Reagent C (GSH)	0.05	0.05

Mix by inversion and equilibrate for 5 minutes at 25°C. Record the initial A_{240nm} for both the Test and Blank using a suitably thermostatted spectrophotometer. Then add:

Reagent E (Glyoxalase I)	0.10	0.10

Mix by inversion and allow the reaction to proceed for 10 minutes. Record the final A_{240nm} for both the Test and Blank.

CALCULATIONS:

$\Delta A_{240nm} = A_{f\ 240nm} - A_{i\ 240nm}$

A_i = Initial Absorbance
A_f = Final Absorbance

$$\text{micromoles Methylglyoxal/weighed sample} = \frac{(\Delta A)(3.00)(df)}{(3.37)(0.5)}$$

3.00 = Total volume of Reaction Mix
df = Dilution factor
3.37 = Millimolar extinction coefficient of S-Lactoylglutathione at 240 nm
0.5 = Volume of Methyglyoxal used in assay

$$\text{Apparent molecular weight} = \frac{\text{mg sample weighed x 1000}}{\mu\text{moles Methylglyoxal/weighed sample}}$$

$$\%\ \text{Methylglyoxal in sample} = \frac{\text{theoretical molecular weight}}{\text{apparent molecular weight}}$$

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FINAL ASSAY CONCENTRATION:

In a 3.0 ml reaction mix, the final concentrations are 34 mM potassium phosphate, 0.033% (w/v) reduced glutathione, 0.03% (w/v) bovine serum albumin, and 0.25 unit of glyoxalase I.

REFERENCE:

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

NOTES:

1. Wear respirator when handling methylglyoxal directly from the bottle.

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