Determination of the Concentration and Molecular Weight of METHYLGLYOXAL

**PRINCIPLE:**

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

Abbreviations:
GSH = Reduced Glutathione

**CONDITIONS:** \( T = 25^\circ C, \ \text{pH} \ 6.6, \ A_{240nm}, \ \text{Light path} = 1 \ cm \)

**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.)

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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>2.25</td>
<td>2.75</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent B (Meth-Glyox)</td>
<td>0.50</td>
<td>------</td>
</tr>
<tr>
<td>Reagent C (GSH)</td>
<td>0.05</td>
<td>0.05</td>
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</tbody>
</table>

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:

\[ r \ A_{240nm} = A_f \ A_{240nm} - A_i \ A_{240nm} \]

\[ A_i = \text{Initial Absorbance} \]

\[ A_f = \text{Final Absorbance} \]

\[ (r \ A) \ (3.00) \ (df) \]

micromoles METHYLGLYOXAL/weighed sample = \[
\frac{(r \ A) \ (3.00) \ (df)}{(3.37) \ (0.5)}
\]

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 METHYLGLYOXAL used in assay

Apparent molecular weight = \[
\frac{\text{mg sample weighed} \times 1000}{\mu \text{moles METHYLGLYOXAL/weighed sample}}
\]

% 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:


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.

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