Determination of the Molecular Weight of TETRAHYDROFOLIC ACID

PRINCIPLE:

\[ \text{THF} + \text{FIGLU} \xrightarrow{\text{FIGLU Transferase}} \text{l-Glutamate} + 5\text{-Formimino-THF} \]

Abbreviations:

THF = Tetrahydrofolic Acid
FIGLU = Formimino-L-Glutamic Acid
5-Formimino-THF = 5-Formimino-Tetrahydrofolic Acid

CONDITIONS: \( T = 25^\circ C, \ \text{pH} = 7.2, \ A_{350\text{nm}}, \ \text{Light path} = 1 \text{ cm} \)

METHOD: Spectrophotometric

REAGENTS:

A. 200 mM Potassium Phosphate Buffer, pH 7.2 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.2 at 25°C with 1 M KOH.)

B. 54 mM Formimino-l-Glutamic Acid Solution (FIGLU)
(Prepare by dissolving 65 mg of Formimino-l-Glutamic Acid, Hemibarium Salt, Sigma Prod. No. F-8626, in 4.5 ml of deionized water. Add 0.50 ml of 0.5 M Sodium Sulfate, Anhydrous, Sigma Prod. No. S-9627 to precipitate Barium Sulfate. Centrifuge and save the supernatant.)

C. Tetrahydrofolic Acid Solution (THF)
(Immediately before use, weigh approximately 4.0 mg of Tetrahydrofolic Acid and dissolve in 10 ml of Reagent D.)

D. 20 mM Potassium Hydroxide Solution with 1000 mM 2-Mercaptoethanol (KOH/2-ME)
(Prepare 25 ml in deionized water using Potassium Hydroxide, Sigma Prod. No. P-1767 and 2-Mercaptoethanol, Sigma Prod. No. M-6250.)

E. Formimino-l-Glutamic Acid Transferase Enzyme Solution (FIGLU Transferase)
(Immediately before use, prepare a solution containing

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0.3 unit/ml of Formimino-L-Glutamic Acid Transferase, Sigma Prod. No. F-0777 in cold deionized water.)
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REAGENTS: (continued)

F. 10% (v/v) Perchloric Acid Solution (Per-Acid)
(Prepare 25 ml in deionized water using Perchloric Acid, Aldrich Stock No. 24425-2.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Substrate</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.50</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Reagent B (FIGLU)</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent C (THF)</td>
<td>0.50</td>
<td>0.50</td>
<td>------</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>1.30</td>
<td>1.50</td>
<td>1.80</td>
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</tbody>
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Mix by inversion and equilibrate for 5 minutes at 25°C. Record the initial \( (A_i, 350\text{nm}) \) for the Test, Enzyme Blank, and Substrate Blank using a suitably thermostatted spectrophotometer. Then add:

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<tbody>
<tr>
<td>Reagent E (FIGLU Transferase)</td>
<td>0.20</td>
<td>------</td>
<td>0.20</td>
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Mix by inversion and allow the reaction to proceed for 1 - 2 hours at 25°C. Then add:

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<tbody>
<tr>
<td>Reagent F (Per Acid)</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
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</table>

Mix by swirling and place in a boiling water bath for 1 minute. Then cool in an ice bath and centrifuge to clarify. Transfer the solutions to suitable cuvettes and record the final \( (A_f, 350\text{nm}) \) for the Test, Enzyme Blank and Substrate Blank using a suitable Spectrophotometer.

CALCULATION:

\[
r_{A_{350\text{nm}}} = A_{f, 350\text{nm}} - A_{i, 350\text{nm}}
\]

Corrected \[
r_{A_{350\text{nm}}} = r_{A_{350\text{nm}}} \text{ Test} - (r_{A_{350\text{nm}}} \text{ Enzyme Blank} + r_{A_{350\text{nm}}} \text{ Substrate Blank})
\]
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**CALCULATIONS:**

\[
\text{Micromoles THF/weighed sample} = \frac{(\text{Corrected } r A_{350\text{nm}}) \times (6.5) \times (10)}{(25) \times (0.5)}
\]

6.5 = Total volume of colorimetric assay
0.5 = Volume of THF used in assay
10 = Dilution factor of weighed sample
25 = Millimolar extinction coefficient of 5,10-methenyltetrahydrofolic Acid at 350 nm
(5-Formimino-THF is converted to 5,10-Methenyltetrahydrofolic Acid under acidic conditions.)

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

**FINAL ASSAY CONCENTRATIONS:**

In a 5.00 ml reaction mix, the final concentrations are:
- 100 mM potassium phosphate
- 5.4 mM FIGLU
- 100 mM 2-mercaptoethanol
- 2.0 mM potassium hydroxide
- 0.06 unit formimino-\text{-}L\text{-}glutamic acid transferase

**REFERENCE:**


**NOTES:**

1. 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.