

**Determination of the Molecular Weight
of TETRAHYDROFOLIC ACID**

PRINCIPLE:

THF + FIGLU $\xrightarrow{\text{FIGLU Transferase}}$ L-Glutamate + 5-Formimino-THF

Abbreviations:

THF = Tetrahydrofolic Acid

FIGLU = Formimino-L-Glutamic Acid

5-Formimino-THF = 5-Formimino-Tetrahydrofolic Acid

CONDITIONS: T = 25°C, pH = 7.2, A_{350nm}, Light path = 1 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

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:

	Enzyme		Subs
	<u>Test</u>	<u>Blank</u>	trate <u>Blank</u>
Reagent A (Buffer)	2.50	2.50	2.50
Reagent B (FIGLU)	0.50	0.50	0.50
Reagent C (THF)	0.50	0.50	-----
Deionized Water	1.30	1.50	1.80

Mix by inversion and equilibrate for 5 minutes at 25°C.
Record the initial ($A_{i, 350nm}$) for the Test, Enzyme Blank,
and Substrate Blank using a suitably thermostatted
spectrophotometer. Then add:

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

Reagent F (Per Acid)	1.50	1.50	1.50
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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 ($A_{f, 350nm}$) for the Test, Enzyme Blank and
Substrate Blank using a suitable Spectrophotometer.

CALCULATION:

$$r A_{350nm} = A_{f, 350nm} - A_{i, 350nm}$$

$$\text{Corrected } r A_{350nm} = r A_{350nm} \text{ Test} - (r A_{350nm} \text{ Enzyme Blank} + r A_{350nm} \text{ Substrate Blank})$$

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CALCULATIONS:

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

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.)

$$\text{Apparent molecular weight} = \frac{\text{mg sample weighed} \times 1000}{\text{umoles of THF/weighed sample}}$$

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 and 0.06 unit formimino-L-glutamic acid transferase.

REFERENCE:

Tabor, H. and Wyngarden, L. (1959) *Journal of Biological Chemistry* **234**, 1830-1846.

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.