**Enzymatic Assay of**
**FORMIMINO-L-GLUTAMIC ACID TRANSFERASE**
**(EC 2.1.2.5)**

**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, $A_{365nm}$, Light path = 1 cm

**METHOD:** Colorimetric

**REAGENTS:**

A. 500 mM Potassium Phosphate Buffer with 500 mM 2-Mercaptoethanol, pH 7.4 at 25°C (Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379 and 2-Mercaptoethanol, Prod. No. M-6250. Adjust to pH 7.4 at 25°C with 1 M KOH.)

B. 5.6 mM Tetrahydrofolic Acid Solution (THF) (Prepare 10 ml in Reagent A using Tetrahydrofolic Acid, Prod. No. T-3125. **PREPARE IMMEDIATELY BEFORE USE**.)

C. 36 mM Formimino-L-Glutamic Acid Solution (FIGLU) (Prepare 10 ml in deionized water using Formimino-L-Glutamic Acid, Hemibarium Salt, Prod. No. F-8626. Add small amounts of Sodium Sulfate, Anhydrous, Prod. No. S-9627 to precipitate barium sulfate. Centrifuge after each sodium sulfate addition. Add sodium sulfate until no precipitate forms. Save the supernatant.)

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

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**REAGENTS:** (continued)

E. Formimino-$_L$-Glutamic Acid Transferase Enzyme Solution (Immediately before use, prepare a solution containing 1.0 - 2.0 units/ml of Formimino-$_L$-Glutamic Acid Transferase in cold deionized water.)

**PROCEDURE:**

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

| | Test | Blank |
|---|---|---|
| Reagent B (THF) | 0.60 | 0.60 |
| Reagent C (FIGLU) | 0.30 | ------ |
| Deionized water | 2.00 | 2.30 |

Mix by inversion and equilibrate to 25°C. Then add:

| | | |
|---|---|---|
| Reagent E (Enzyme Solution) | 0.010 | 0.010 |

Mix by inversion and incubate at 25°C for exactly 5 minutes. Then add:

| | | |
|---|---|---|
| Reagent D (Per Acid) | 1.0 | 1.0 |

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 $A_{365nm}$ for both the Test and Blank using a suitable spectrophotometer.

**CALCULATION:**

$$\text{Units/mg enzyme} = \frac{(A_{365nm}\ \text{Test} - A_{365nm}\ \text{Blank})\ (3.91)}{(5)\ (22.1)(\text{mg enzyme/RM})}$$

5 = Time of assay (in minutes) as per unit definition
22.1 = Millimolar extinction coefficient of 5,10-Methenyltetrahydrofolic Acid at 365 nm (5-Formimino-THF is converted to 5,10-Methenyltetrahydrofolic Acid under acidic conditions)
3.91 = Volume of colorimetric assay
RM = Reaction Mix

# Enzymatic Assay of FORMIMINO-L-GLUTAMIC ACID TRANSFERASE (EC 2.1.2.5)

## UNIT DEFINITION:

One unit will convert 1.0 µmole of FIGLU and THF to L-glutamic acid and 5-formimino-THF per minute at pH 7.2 at 25°C (measured as 5,10-methenyl-THF after perchloric acid treatment.)

## FINAL ASSAY CONCENTRATION:

In a 2.91 ml reaction mix, the final concentrations are 103 mM potassium phosphate, 103 mM 2-mercaptoethanol, 1.2 mM THF, 3.7 mM FIGLU, and 0.010 - 0.020 unit formimino-L-glutamic acid transferase.

## REFERENCES:

Tabor, H. and Wyngarden, L. (1958) *Journal of Clinical Investigation* **37**, 824-828.

## NOTES:

1. This assay is a modification of the assay described in the cited reference.

2. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.