Enzymatic Assay of
FORMIMINO-\textit{L}-GLUTAMIC ACID TRANSFERASE  
(EC 2.1.2.5)

\textbf{PRINCIPLE:}

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

\textbf{Abbreviations:}
THF = Tetrahydrofolic Acid  
FIGLU = Formimino-\textit{L}-Glutamic Acid  
5-Formimino-THF = 5-Formimino-Tetrahydrofolic Acid

\textbf{CONDITIONS: } T = 25^\circ \text{C}, \text{pH} = 7.2, A_{\lambda=365nm}, \text{Light path} = 1 \text{ cm}

\textbf{METHOD:} Colorimetric

\textbf{REAGENTS:}

A. 500 mM Potassium Phosphate Buffer with 500 mM 2-Mercaptoethanol, pH 7.4 at 25^\circ \text{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^\circ \text{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. \textbf{PREPARE IMMEDIATELY BEFORE USE.})

C. 36 mM Formimino-\textit{L}-Glutamic Acid Solution (FIGLU)  
(Prepare 10 ml in deionized water using Formimino-\textit{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-\textit{L}-Glutamic Acid Transferase Enzyme Solution
(Immediately before use, prepare a solution containing
1.0 - 2.0 units/ml of Formimino-\textit{L}-Glutamic Acid
Transferase in cold deionized water.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (THF)</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>Reagent C (FIGLU)</td>
<td>0.30</td>
<td>------</td>
</tr>
<tr>
<td>Deionized water</td>
<td>2.00</td>
<td>2.30</td>
</tr>
</tbody>
</table>

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

CALCULATION:

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

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
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UNIT DEFINITION:

One unit will convert 1.0 µmole of FIGLU and THF to 
\text-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-\text-L-glutamic acid transferase.

REFERENCES:

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.

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