Enzymatic Assay of \textit{L}\text{-METHIONINE GAMMA-LYASE}
(EC 4.4.1.11)

PRINCIPLE:

\[
\begin{align*}
\text{L-Methionine Gamma-Lyase} \\
\text{L-Methionine} & \xrightarrow{} \text{Methanethiol + 2-Ketobutyrate + NH}_3 \\
\text{2-Ketobutyrate + MBTH} & \xrightarrow{} \text{Azine Derivative}
\end{align*}
\]

Abbreviation used:

MBTH = 3-Methyl-2-Benzothiazolinone

CONDITIONS: \( T = 37^\circ C, \ \text{pH} = 8.0, \ A_{320nm}, \ \text{Light path} = 1 \ \text{cm} \)

METHOD: Stopped Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer with 25 mM \textit{L}\text{-Methionine and 0.01 mM Pyridoxal 5-Phosphate, pH 8.0 at 37°C} \textsuperscript{\dagger}

(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, \textit{L-Methionine}, and Pyridoxal 5-Phosphate. Adjust to pH 8.0 at 37°C with 5 M KOH.)

B. 50\% (w/v) Trichloroacetic Acid Solution (TCA)

(Prepare 5 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100\% (w/v))

C. 1 M Sodium Acetate Buffer, pH 5.0 at 37°C (NaOAC)

(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate. Adjust to pH 5.0 at 37°C with 5 M HCl.)

D. 0.1\% (w/v) 3-Methyl-2-Benzothiazolinone Hydrazone (MBTH)

(Prepare 10 ml in deionized water using 3-Methyl-2-Benzothiazolinone Hydrazone, Hydrochloride Hydrate.)
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REAGENTS:

E. 100 mM Potassium Phosphate Buffer with
   1 mM Ethylenediaminetetraacetic Acid, 0.01% (v/v) 2-Mercaptoethanol and 0.02 mM Pyridoxal
   5-Phosphate, pH 7.2 at 37°C (Enzyme Diluent)
   (Prepare 10 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, 2-Mercaptoethanol,
   Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, and Pyridoxal 5-Phosphate.
   Adjust to pH 7.2 at 37°C with 5 M KOH. Store on ice.)

F. L-Methionine Gamma-Lyase Enzyme Solution
   (Immediately before use, prepare a solution containing 0.08 - 0.4 unit/ml of L-Methionine
   Gamma-Lyase in ice-cold Reagent E.)

PROCEDURE:

Step 1:

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>Reagent A (Buffer)</td>
<td>2.00</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Equilibrate to 37°C. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>0.02</td>
<td>-----</td>
</tr>
<tr>
<td>Reagent E (Enzyme Diluent)</td>
<td>-----</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Mix by inversion and incubate at 37°C for exactly 10 minutes. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (TCA)</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Mix by inversion.
Enzymatic Assay of L-METHIONINE GAMMA-LYASE  
(EC 4.4.1.11)

PROCEDURE: (continued)

Step 2:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (NaOAC)</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Test Reaction Mix from Step 1</td>
<td>1.00</td>
<td>-----</td>
</tr>
<tr>
<td>Blank Reaction Mix from Step 1</td>
<td>-----</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent D (MBTH)</td>
<td>0.80</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Mix by inversion and incubate at 50°C for exactly 30 minutes. Then incubate at 25°C for 30 minutes.

Transfer the Test and Blank solutions to suitable cuvettes and record the A$_{320nm}$ using a suitable spectrophotometer.

CALCULATION:

\[
\text{Units/vial enzyme} = \frac{(A_{320nm} \text{ Test} - A_{320nm} \text{ Blank})(2.27)(3.8)(df)}{15.74(10)(0.02)(1)} 
\]

2.27 = Volume (in milliliters) of assay in Step 1
3.8 = Volume (in milliliters) of assay in Step 2
df = Dilution factor
15.74 = Millimolar extinction coefficient of the azine derivative
10 = Time (in minutes) of assay as per the Unit Definition
0.02 = Volume (in milliliter) of enzyme used in Step 1
1 = Volume (in milliliter) of Step 1 used in Step 2

UNIT DEFINITION:

One unit will release 1.0 micromole of alpha-ketobutyrate from L-methionine per minute at pH 8.0 at 37°C.

REFERENCE:

In a 2.02 ml reaction mix, the final concentrations are 100 mM potassium phosphate, 25 mM L-methionine, 0.01 mM pyridoxal 5-phosphate, 0.0001% (v/v) 2-mercaptoethanol, 0.01 mM ethylenediaminetetraacetic acid, and 0.0016 - 0.008 unit L-methionine gamma-lyase.
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REFERENCE:


Soda, K. (1968) Analytical Biochemistry 25, 228-235

NOTES:

1. This reagent may be prepared by first making stock solutions of the separate components and then combining them. This is necessary since extremely small weigh-ups are required.

2. This assay is based on the cited references.

3. Where Products are specified, equivalent reagents may be substituted.

This procedure is for informational purposes.