

Enzymatic Assay of L-METHIONINE GAMMA-LYASE (EC 4.4.1.11)

PRINCIPLE:

L-Methionine $\xrightarrow{\text{L-Methionine Gamma-Lyase}}$ Methanethiol + 2-Ketobutyrate + NH₃

2-Ketobutyrate + MBTH \longrightarrow Azine Derivative

Abbreviation used:

MBTH = 3-Methyl-2-Benzothiazolinone

CONDITIONS: T = 37°C, pH = 8.0, A_{320nm}, Light path = 1 cm

METHOD: Stopped Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Potassium Phosphate Buffer with 25 mM L-Methionine and 0.01 mM Pyridoxal 5-Phosphate, pH 8.0 at 37°C¹
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, L-Methionine, Sigma Prod. No. M-9625, and Pyridoxal 5-Phosphate, Sigma Prod. No. P-9255. 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), Sigma Stock No. 490-10.)
- C. 1 M Sodium Acetate Buffer, pH 5.0 at 37°C (NaOAc)
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. 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, Sigma Prod. No. M-8006.)

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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)¹
(Prepare 10 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma
Prod. No. P-5379, 2-Mercaptoethanol, Sigma Prod. No. M-6250, Ethylenediaminetetraacetic
Acid, Tetrasodium Salt, Hydrate, Sigma Stock No. ED4SS, and Pyridoxal 5-Phosphate,
Sigma Prod. No. P-9255. 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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.00	2.00

Equilibrate to 37°C. Then add:

Reagent F (Enzyme Solution)	0.02	-----
Reagent E (Enzyme Diluent)	-----	0.02

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

Reagent B (TCA)	0.25	0.25
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Mix by inversion.

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PROCEDURE: (continued)

Step 2:

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

	<u>Test</u>	<u>Blank</u>
Reagent C (NaOAC)	2.00	2.00
Test Reaction Mix from Step 1	1.00	-----
Blank Reaction Mix from Step 1	-----	1.00
Reagent D (MBTH) 0.80	0.80	

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:

Tanaka, H.I., Imahara, H., Esaki, N., and Soda, K. (1980) *Journal of Applied Biochemistry* **2**, 439-444

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 Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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