SIGMA QUALITY CONTROL TEST

ProductInformation

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

PRINCIPLE:

L-Methionine $\xrightarrow{\text{L-Methionine Gamma-Lyase}}$ Methanethiol + 2-Ketobutyrate + NH_3

2-Ketobutyrate + MBTH ⟶ 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°C[1]
(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:

	Test	Blank
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

Mix by inversion.

PROCEDURE: (continued)

Step 2:

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

	Test	Blank
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.