Enzymatic Assay of LYSINE OXIDASE
(EC 1.4.3.14)

PRINCIPLE:

$_L$-Lysine + O_2 + H_2O $\xrightarrow{\text{Lysine Oxidase}}$ 6-Amino-2-Oxohexanoate + NH_3 + H_2O_2

H_20_2 + 6-Amino-2-Oxohexanoate $\xrightarrow{\text{Catalase}}$?^{1}-Piperidine-2-Carboxylate

?^{1}-Piperidine-2-Carboxylate $\xrightarrow{\text{MBTH}}$ UV absorbing complex

Abbreviation used:
MBTH = 3-Methyl-2-Benzothiazolinone Hydrazone

CONDITIONS: T = 37°C, pH = 8.0, A_{317nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 100 mM $_L$-Lysine Solution ($_L$-Lys)
(Prepare 5 ml in deionized water using $_L$-Lysine Monohydrochloride, Sigma Prod. No. L-5626.)

B. 200 mM Potassium Phosphate Buffer, pH 8.0 at 37°C (Buffer I)
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 8.0 at 37°C with 1 M KOH.)

C. 0.1% (w/v) 3-Methyl-2-Benzothialinone Hydrazone Solution (MBTH)
(Prepare 10 ml in deionized water using 3-Methyl-2-Benzothiazolinone Hydrazone, Hydrochloride, Hydrate, Sigma Prod. No. M-8006. This reagent is **TOXIC**. **PREPARE FRESH**.)

D. 12.5% (w/v) Trichloroacetic Acid Solution (TCA)
(Prepare 2 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)

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REAGENTS: (continued)

E. 10 mM a-Ketobutyric Acid Solution (a-KA)
(Prepare 2 ml in deionized water using a-Ketobutyric Acid, Free Acid, Sigma Prod. No. K-8875.)

F. 1 M Sodium Acetate Buffer, pH 5.0 at 37°C (Buffer II)
(Prepare 50 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.0 at 37°C with 1 M HCl.)

G. Catalase Enzyme Solution (Catalase)
(Immediately before use, prepare a solution containing 3,500 units/ml in cold deionized water using Catalase, Sigma Prod. No. C-9322.)

H. Lysine Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.03 - 0.1 unit/ml in cold deionized water.)

PROCEDURE:

Step 1

Pipette (in milliliters) the following reagents into an Eppendorf tube:

	Test	Blank
Reagent A (L-Lys)	0.10	0.10
Reagent B (Buffer I)	0.35	0.35
Reagent G (Catalase)	0.10	0.10
Deionized Water	0.35	0.45

Equilibrate at 37°C for 2 minutes. Then add:

Reagent H (Lysine Oxidase)	0.10	------

Immediately mix by inversion and incubate at 37°C for exactly 20 minutes. Then add:

Reagent D (TCA)	0.10	0.10

Centrifuge the Test and Blank for 5 minutes.

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PROCEDURE: (continued)

Step II

Colorimetric Assay:

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

	Test	Blank	Std 1	Std 2	Std 3	Std 4	Std 5	Std Blank
Reagent C (MBTH)	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Reagent F (Buffer II)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Test Supernatant	0.50	----	----	----	----	----	----	----
Blank Supernatant	----	0.50	----	----	----	----	----	----
Reagent E (a-KA)	----	----	0.02	0.05	0.10	0.15	0.25	----
Deionized Water	1.10	1.10	1.58	1.55	1.50	1.45	1.35	1.60

Mix by swirling and incubate at 50°C for exactly 30 minutes. Cool to room temperature and record the A_{317nm} for the Test, Blank, Standards, and Standard Blank, using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

$?A_{317nm}$ Standard = A_{317nm} Standard - A_{317nm} Standard Blank

Prepare a standard curve by plotting the A_{317nm} Standard versus micromoles of a-ketobutyric acid.

Sample Determination:

r A_{317nm} Test = A_{317nm} Test - A_{317nm} Test Blank

Determine the micromoles of a-keto acid equivalents formed using the standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of a-keto acid equivalents formed})(1.1)(df)}{(0.5)(20)(0.1)}$$

1.1 = Volume (in milliliter) of stopped assay

df = Dilution factor

CALCULATIONS: (continued)

0.5 = Volume (in milliliter) of stopped assay used in the colorimetric assay
20 = Time (in minutes) of assay
0.1 = Volume (in milliliter) of L-lysine oxidase used in the assay

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will catalyze the formation of 1 µmole of 6-amino-2-oxohexanoic acid from L-lysine per minute at 37°C at pH 8.0.

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 10 mM L-lysine, 70 mM potassium phosphate, 350 units catalase, and 0.003 - 0.01 unit L-lysine oxidase.

REFERENCE:

Kusakabe, H., Kodama, K., Yoshino, H. Kuninaka, A., H., Misono, H., and Soda, K. (1980) *Journal of Biological Chemistry* **255**, 976-981

NOTES:

1. Catalase Unit Definition: One unit will decompose 1.0 µmole of H_2O_2 per minute at pH 7.0 at 25°C, while the H_2O_2 concentration falls from 10.3 to 9.2 mM. The rate of disappearance of H_2O_2 is followed by observing the rate of decrease in absorbance at 240 nm.

2. This assay is based on the cited reference.

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