Enzymatic Assay of L-GLUTAMATE OXIDASE
(EC 1.4.3.11)

PRINCIPLE:

$$\text{L-Glutamate} + O_2 + H_2O \xrightarrow{\text{L-Glutamate Oxidase}} \text{a-Ketoglutaric Acid} + NH_3 + H_2O_2$$

$$2\ H_2O_2 + H_2O \xrightarrow{\text{Catalase}} 2\ H_2O + O_2$$

CONDITIONS: T = 30°C, pH = 7.4, A_{316nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 7.4 at 30°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.4 at 30°C with 1 M KOH.)

B. 100 mM L-Glutamate Solution (L-Glu)
(Prepare 2 ml in deionized water using L-Glutamic Acid, Monosodium Salt, Sigma Prod. No. G-1626.)

C. 25% (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.)

D. 1 M Acetate Buffer, pH 5.0 at 37°C (Acetate)
(Prepare 25 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.)

E. 0.10% (w/v) 3-Methyl-2-Benzothiazolinone Hydrazone Solution (MBTH)
(Prepare 10 ml in deionized water using 3-Methyl-2-Benzothiazolinone Hydrazone, Hydrochloride, Hydrate, Sigma Prod. No. M-8006.)

F. 2.6 mM a-Ketoglutaric Acid Solution (KG)
(Prepare 1 ml in Reagent A using a-Ketoglutaric Acid, Disodium Salt, Sigma Prod. No. K-3752.)

REAGENTS: (continued)

G. Catalase Enzyme Solution (Catalase)
(Immediately before use, prepare a solution containing 300 units/ml of Catalase, Sigma Prod. No. C-9322, in cold deionized water.)

H. L-Glutamate Oxidase Enzyme Solution (L-Glu Ox)
(Immediately before use, prepare a solution containing 0.05 - 0.10 unit/ml of L-Glutamate Oxidase in cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	0.70	0.80
Reagent G (Catalase)	0.10	0.10
Reagent B (L-Glu)	0.10	0.10

Mix by swirling and equilibrate to 30°C. Then add:

	Test	Blank
Reagent H (L-Glu Ox)	0.10	------

Immediately mix by swirling and incubate at 30°C for exactly 20 minutes with shaking. Then add:

	Test	Blank
Reagent C (TCA)	0.10	0.10
Reagent D (Acetate)	1.90	1.90
Reagent E (MBTH)	0.80	0.80

Mix by swirling and incubate at 50°C for 30 minutes. Then allow to stand at room temperature for 20 minutes. Transfer the solutions to suitable cuvettes and record the A_{316nm} for both the Test and Blank using a suitable spectrophotometer.

COLORIMETRIC ASSAY:

Standard Curve:

Prepare a standard curve by pipetting (in milliliters) the following reagents into suitable containers:

	Std 1	Std 2	Std 3	Std 4	Std 5	Std Blank
Reagent F (KG)	0.02	0.04	0.06	0.08	0.10	------

Reagent A (Buffer)	0.98	0.96	0.94	0.92	0.90	1.00

COLORIMETRIC ASSAY: (continued)

Mix by swirling and incubate at 30°C for exactly 20 minutes with shaking. Then add:

	Std 1	Std 2	Std 3	Std 4	Std 5	Std Blank
Reagent C (TCA)	0.10	0.10	0.10	0.10	0.10	0.10
Reagent D (Acetate)	1.90	1.90	1.90	1.90	1.90	1.90
Reagent E (MBTH)	0.80	0.80	0.80	0.80	0.80	0.80

Mix by swirling and incubate at 50°C for 30 minutes. Then allow to stand at room temperature for 20 minutes. Transfer the solutions to suitable cuvettes and record the A_{316nm} for the Standards and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

r A_{316nm} Standard = A_{316nm} Standard - A_{316nm} Standard Blank

Prepare a standard curve by plotting the ?A_{316nm} for the Standard versus µmoles of a-Ketoglutarate.

Sample Determination:

r A_{316nm} Sample = A_{316nm} Test - A_{316nm} Blank

Determine the µmoles of a-Ketoglutarate produced using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of a-Ketoglutarate produced})(\text{df})}{(20)(0.1)}$$

df = Dilution factor
20 = Time (in minutes) of assay as per the Unit Definition
0.1 = Volume (in milliliter) of enzyme used in assay

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

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

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UNIT DEFINITION:

One unit will form 1.0 µmole of a-ketoglutaric acid from L-glutamic acid per minute at pH 7.4 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 80 mM potassium phosphate, 10 mM L-glutamic acid, 30 units catalase, and 0.005 - 0.01 unit L-glutamate oxidase.

REFERENCES:

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

Kusakabe, H., Midorikawa, Y., Kuninaka, A., and Yoshino, H. (1983) *Agricultural Biological Chemistry* **47**, 179-182

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.

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.