Enzymatic Assay of L-GLUTAMIC DEHYDROGENASE (NADP) (EC 1.4.1.4)

PRINCIPLE:

$$\text{a-KG} + \text{ß-NADPH} + NH_4^+ \xrightarrow{\text{L-Glutamic Dehydrogenase}} \text{L-Glu} + \text{ß-NADP} + H_2O$$

Abbreviations used:
a-KG = a-Ketoglutarate
L-Glu = L-Glutamate
ß-NADPH = ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
ß-NADP = ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form

CONDITIONS: T = 30°C, pH = 8.3, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Tris HCl Buffer, pH 8.3 at 30°C
(Prepare 100 ml in deionized water using Trizma, Hydrochloride, Sigma Prod. No. T-3253. Adjust to pH 8.3 at 30°C with 1 M NaOH.)

B. 225 mM a-Ketoglutarate Solution (a-KG)
(Prepare 10 ml in Reagent A using a-Ketoglutaric Acid, Sigma Prod. No. K-1750 or a-Ketoglutaric Acid, Disodium Sigma Prod. No. K-3752. Adjust to pH 7.0 - 9.0 at 30°C with 1 M NaOH.)

C. 3300 mM Ammonium Chloride Solution (NH_4Cl)
(Prepare 5 ml in deionized water using Ammonium Chloride,
Sigma Prod. No. A-4514.)

D. 7.5 mM ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form Solution (ß-NADPH)
(Prepare 1 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form, Tetrasodium Salt, Sigma Prod. No. N-1630 or equivalent. **PREPARE FRESH**.)

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REAGENTS: (continued)

E. 50 mM Potassium Phosphate with 50 mM Ethylenediaminetetraacetic Acid Solution, pH 6.6 at 30°C (Enzyme Diluent)
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504 and Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS. Adjust to pH 6.6 at 30°C with 1 M KOH.)

F. L-Glutamic Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of L-Glutamic Dehydrogenase in cold Reagent E.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.50	2.50
Reagent B (a-KG)	0.10	0.10
Reagent C (NH_4Cl)	0.20	0.20
Reagent D (ß-NADPH)	0.10	0.10

Mix by inversion and equilibrate to 30°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent F (Enzyme Solution)	0.10	---
Reagent E (Enzyme Diluent)	------	0.10

Immediately mix by inversion and record the decrease in A_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3)(df)}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADPH at 340 nm
0.1 = Volume (in milliliters) of enzyme used

CALCULATIONS: (continued)

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

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

UNIT DEFINITION:

One unit will reduce 1.0 µmole of a-ketoglutarate to L-glutamate per minute at pH 8.3 at 30°C, in the presence of ammonium ions and NADPH.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 83 mM Tris, 7.5 mM a-ketoglutarate, 220 mM ammonium chloride, 0.25 mM ß-nicotinamide adenine dinucleotide phosphate, reduced form, 1.7 mM potassium phosphate, 1.7 mM ethylenediaminetetraacetic acid, and 0.03 - 0.06 unit L-glutamic dehydrogenase.

REFERENCE:

Shimizu, H., Kuratsu, T., and Hirata, F. (1979) *J. Ferment. Technol.* **57**, 428-433

NOTES:

1. This assay is a modification of the assay described in the cited reference.

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