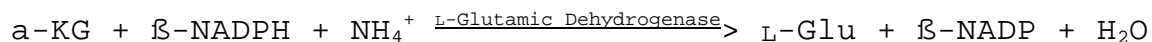


**Enzymatic Assay of L-GLUTAMIC DEHYDROGENASE (NADP)  
(EC 1.4.1.4)**

**PRINCIPLE:**



Abbreviations used:

$\alpha$ -KG =  $\alpha$ -Ketoglutarate

L-Glu = L-Glutamate

$\beta$ -NADPH =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Reduced Form

$\beta$ -NADP =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Oxidized Form

**CONDITIONS:** T = 30°C, pH = 8.3, A<sub>340nm</sub>, 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  $\alpha$ -Ketoglutarate Solution ( $\alpha$ -KG)  
(Prepare 10 ml in Reagent A using  $\alpha$ -Ketoglutaric Acid, Sigma Prod. No. K-1750 or  $\alpha$ -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<sub>4</sub>Cl)  
(Prepare 5 ml in deionized water using Ammonium Chloride, Sigma Prod. No. A-4514.)
- D. 7.5 mM  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form Solution ( $\beta$ -NADPH)  
(Prepare 1 ml in deionized water using  $\beta$ -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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.50	
		2.50
Reagent B (a-KG)	0.10	0.10
Reagent C (NH <sub>4</sub> Cl)	0.20	0.20
Reagent D (β-NADPH)	0.10	0.10

Mix by inversion and equilibrate to 30°C. Monitor the A<sub>340nm</sub> 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<sub>340nm</sub> for approximately 5 minutes. Obtain the r A<sub>340nm</sub>/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

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

3 = Total volume (in milliliters) of assay

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

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**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  $\mu$ mole of  $\alpha$ -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  $\alpha$ -ketoglutarate, 220 mM ammonium chloride, 0.25 mM  $\beta$ -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.

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