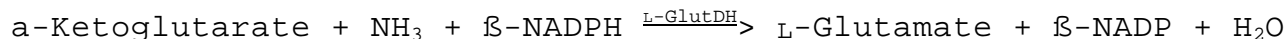


**Enzymatic Assay of L-GLUTAMIC DEHYDROGENASE (β -NADP)
(EC 1.4.1.4)**

PRINCIPLE:



Abbreviations used:

β -NADPH = β -Nicotinamide Adenine Dinucleotide Phosphate,
Reduced Form

β -NADP = β -Nicotinamide Adenine Dinucleotide Phosphate

L-GlutDH = L-Glutamic Dehydrogenase

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, Prod. No. T-3253. Adjust to pH 8.3 at 30°C with 1 M NaOH.)
- B. 3.3 M Ammonium Chloride Solution (NH₄Cl)
(Prepare 2 ml in deionized water using Ammonium Chloride, Prod. No. A-4514.)
- C. 225 mM a-Ketoglutarate Solution (a-KG)
(Prepare 5 ml in deionized water using a-Ketoglutaric Acid, Free Acid, Prod. No. K-1750. Adjust to pH 8 with 1 M NaOH.)
- D. 7.5 mM β -Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form Solution (β -NADPH)
(Dissolve the contents of one 10 mg vial of β -Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form, Tetrasodium Salt, Stock. No. 201-210, in 1.6 ml of deionized water **or** prepare 1 ml in deionized water using β -Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form, Tetrasodium Salt, Prod. No. N-1630. **PREPARE FRESH.**)

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REAGENTS: (continued)

- E. 50 mM Potassium Phosphate with 50 mM Ethylenediamine-tetraacetic Acid Solution, pH 6.6 at 30°C
(Prepare 20 ml in deionized water using Potassium Phosphate, Monobasic, Prod. No. P-5379, and Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Stock No. ED4SS. Adjust to pH 6.6 at 30°C using 1 M NaOH.)
- F. L-Glutamic Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.3 - 1.0 units/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 (NH ₄ Cl)	0.20	0.20
Reagent C (a-KG)	0.10	0.10
Reagent D (β -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 E (Diluent)	-----	0.05
Reagent F (Enzyme Solution)	0.05	-----

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

CALCULATION:

$$\text{Units/mg enzyme} = \frac{r_{A_{340\text{nm}}/\text{min Test}} - r_{A_{340\text{nm}}/\text{min Blank}}}{(6.22) (\text{mg enzyme/ml RM})}$$

6.22 = Millimolar extinction coefficient of β -NADPH at 340 nm
RM = Reaction Mix

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UNIT DEFINITION:

One unit will reduce 1.0 μ mole of α -ketoglutarate to L-glutamate per minute at pH 8.3 at 30°C in the presence of ammonium ions and β -NADPH.

FINAL ASSAY CONCENTRATION:

In a 2.95 ml reaction mix, the final concentrations are 83 mM Tris HCl, 112 mM ammonium chloride, 7.7 mM α -ketoglutarate, 0.25 mM β -NADPH, 0.83 mM potassium phosphate, 0.83 mM EDTA and 0.015 - 0.05 units L-glutamic dehydrogenase.

REFERENCES:

Shimizu, H. et al. (1979) *J. Ferment. Technol.* **57**, 428.

NOTE:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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