

**SIGMA QUALITY CONTROL TEST  
PROCEDURE****Product Information****Enzymatic Assay of L-GLUTAMIC DEHYDROGENASE  
(EC 1.4.1.3)****PRINCIPLE:**

Abbreviations used:

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

L-Glu = L-Glutamate

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

**CONDITIONS:** T = 25°C, pH = 7.3, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 100 mM Triethanolamine Buffer, pH 7.3 at 25°C  
(Prepare 100 ml in deionized water using Triethanolamine Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.3 at 25°C with 1 M NaOH.)
- B. 200 mM  $\alpha$ -Ketoglutarate Solution ( $\alpha$ -KG)  
(Prepare 10 ml in deionized water using  $\alpha$ -Ketoglutaric Acid, Prod. No. K-1750. Adjust to pH 6.5 - 7.5 using solid Sodium Bicarbonate, Sigma Prod. No. S-8875.)
- C. 3.2 M Ammonium Acetate Solution ( $\text{NH}_4\text{OAc}$ )  
(Prepare 5 ml in deionized water using Ammonium Acetate, Sigma Prod. No. A-7262.)
- D. 10 mM  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form Solution ( $\beta$ -NADH)  
(Prepare 1 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129. **PREPARE FRESH.**)

## Enzymatic Assay of L-GLUTAMIC DEHYDROGENASE (EC 1.4.1.3)

### REAGENTS: (continued)

- E. 25 mM Ethylenediaminetetraacetic Acid Solution (EDTA)  
(Prepare 1 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Sigma Stock No. ED4SS.)
- 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 A.)

### PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable containers.

|                                       |      |
|---------------------------------------|------|
| Reagent A (Buffer)                    | 26.0 |
| Reagent B ( $\alpha$ -KG)             | 2.0  |
| Reagent C ( $\text{NH}_4\text{OAc}$ ) | 0.5  |
| Reagent D ( $\beta$ -NADH)            | 0.3  |
| Reagent E (EDTA)                      | 0.3  |

Mix by stirring and equilibrate to 25°C. Adjust to pH 7.3 at 25°C with 1 M NaOH or 1 M HCl. Then add:

|                   | <u>Test</u> | <u>Blank</u> |
|-------------------|-------------|--------------|
| Reaction Cocktail | 2.90        | 2.90         |

Equilibrate to 25°C. Then add:

|                             |       |       |
|-----------------------------|-------|-------|
| Reagent F (Enzyme Solution) | 0.10  | ----- |
| Reagent A (Buffer)          | ----- | 0.10  |

Immediately mix by inversion and record the decrease in  $A_{340\text{nm}}$  for approximately 5 - 10 minutes. Obtain the  $\Delta A_{340\text{nm}}$ /minute using the maximum linear rate for both the Test and Blank.

### CALCULATIONS:

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

## Enzymatic Assay of L-GLUTAMIC DEHYDROGENASE (EC 1.4.1.3)

### **CALCULATIONS:** (continued)

3 = Volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of  $\beta$ -NADH at 340 nm

0.1 = Volume (in milliliter) of enzyme used

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

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

### **UNIT DEFINITION:**

One unit will reduce 1.0  $\mu$ mole of  $\alpha$ -ketoglutarate to L-glutamate per minute at pH 7.3 at 25°C, in the presence of ammonium ions.

### **FINAL ASSAY CONCENTRATION:**

In a 3.00 ml reaction mix, the final concentrations are 90 mM triethanolamine hydrochloride, 13 mM  $\alpha$ -ketoglutarate, 53 mM ammonium acetate, 0.06 mM  $\beta$ -NADH, 0.25 mM EDTA and 0.03 - 0.06 unit L-glutamic dehydrogenase.

### **NOTES:**

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

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