SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of L-GLUTAMIC DEHYDROGENASE (EC 1.4.1.3)

PRINCIPLE:

$$\alpha\text{-KG} + \beta\text{-NADH} + NH_4^+ \xrightarrow{\text{L-Glutamic Dehydrogenase}} \text{L-Glu} + \beta\text{-NAD} + H_2O$$

Abbreviations used:
α-KG = α-Ketoglutarate
L-Glu = L-Glutamate
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 25°C, pH = 7.3, A_{340nm}, 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 α-Ketoglutarate Solution (α-KG)
(Prepare 10 ml in deionized water using α-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 (NH_4OAc)
(Prepare 5 ml in deionized water using Ammonium Acetate, Sigma Prod. No. A-7262.)

D. 10 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)
(Prepare 1 ml in deionized water using β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129. **PREPARE FRESH.**)

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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 (α-KG)	2.0
Reagent C (NH_4OAc)	0.5
Reagent D (β-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:

	Test	Blank
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_{340nm} for approximately 5 - 10 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

CALCULATIONS: (continued)

3 = Volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-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 µmole of α-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 α-ketoglutarate, 53 mM ammonium acetate, 0.06 mM β-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.