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谷胱甘肽还原酶酶活测试

PRINCIPLE:

β-NADPH + GSSG $\xrightarrow{\text{Glutathione Reductase}}$ β-NADP + 2 GSH

Abbreviations used:
β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
GSSG = Glutathione, Oxidized Form
GSH = Glutathione, Reduced Form

CONDITIONS: T = 25°C, pH = 7.6, A_{340nm}, Light Path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer with 3.4 mM Ethylenediaminetetraacetic Acid (EDTA),pH 7.6 at 25°C(Prepare 200 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, and Ethylenediaminetetraacetic Acid, Dipotassium Salt, Adjust to pH 7.6 at 25°C with 1 M KOH.)

B. 30 mM Glutathione Substrate Solution (GSSG)

(Prepare 5 ml in deionized water using Glutathione, Oxidized Form, Disodium Salt.)

C. 0.8 mM β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form Solution (β-NADPH)(Prepare 5 ml in cold Reagent A using β-Nicotinamide Adenine Dinucleotide Phosphate,Tetrasodium Salt.)

D. 1.0% (w/v) Bovine Serum Albumin (BSA)

(Prepare 100 ml in Reagent A using Albumin, Bovine. This solutionshould be kept cold.)

E. Glutathione Reductase Enzyme Solution

(Immediately before use, prepare a solution containing 0.30 - 0.60 unit/ml of Glutathione Reductase in cold Reagent D.)

PROCEDURE:

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

	Test	Blank
Deionized Water	0.65	0.65
Reagent A (Buffer)	1.50	1.50
Reagent B (GSSG)	0.10	0.10
Reagent C (β-NADPH)	0.35	0.35
Reagent D (BSA)	0.30	0.40

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

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

Immediately mix by inversion and record the decrease in the 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 β-NADPH at 340 nm
0.1 = Volume (in milliliters) 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 oxidized glutathione per minute at pH 7.6 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 75 mM potassium phosphate, 2.6 mM ethylenediaminetetraacetic acid, 1 mM glutathione, 0.09 mM β-nicotinamide adenine dinucleotide phosphate, reduced form, 0.13% (w/v) bovine serum albumin, and 0.03 - 0.06 unit of glutathione reductase.