Enzymatic Assay of GLUTATHIONE-S-TRANSFERASE
(EC 2.5.1.18)
p-Nitrobenzyl Chloride as Substrate

PRINCIPLE:

G-SH + PNBC $\xrightarrow{\text{Glutathione-S-Transferase}}$ G-PNBC Conjugate

Abbreviations:
G-SH = Glutathione, Reduced Form
PNBC = p-Nitrobenzyl Chloride
G-PNBC Conjugate = Glutathione p-Nitrobenzyl Chloride Conjugate

CONDITIONS: T = 25°C, pH = 6.5, A_{310nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer with 1.0 mM Ethylenediaminetetraacetic Acid, pH 6.5 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379, and Ethylenediaminetetraacetic Acid, Tetrasodium Salt: Hydrate, Stock No. ED4S. Adjust to pH 6.5 at 25°C with 1 M KOH.)

B. 150 mM Glutathione, Reduced Solution (G-SH)
(Prepare 10 ml in Reagent A using Glutathione, Free Acid, Reduced Form, Prod. No. G-4251. **PREPARE FRESH**.)

C. 30 mM p-Nitrobenzyl Chloride Solution (PNBC)
(Prepare 1 ml in Reagent D using p-Nitrobenzyl Chloride, Prod. No. N-5634. **PREPARE FRESH**.)

D. 95% Ethanol (Nondenatured)

E. Glutathione-S-Transferase Enzyme Solution
(Immediately before use, prepare a solution containing 0.4-0.8 units/ml of Glutathione-S-Transferase in cold Reagent A.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.70	2.80
Reagent B (G-SH)	0.10	0.10
Reagent C (PNBC)	0.10	0.10

Equilibrate to 25°C. Monitor the A_{310nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

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

Immediately mix by inversion and record the increase in A_{310nm} for approximately 5 minutes. Obtain the ΔA_{310nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{310nm}/\text{min Test} - \Delta A_{310nm}/\text{min Blank})(3)(df)}{(1.9)(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
1.9 = Millimolar extinction coefficient of Glutathione-p-Nitrobenzyl Conjugate at 310 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 conjugate 1.0 µmole of p-nitrobenzyl chloride with reduced glutathione per minute at pH 6.5 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 97 mM potassium phosphate, 0.97 mM EDTA, 5.0 mM glutathione, reduced, 1.0 mM p-nitrobenzyl chloride, 3.2% (v/v) ethanol and 0.04 - 0.08 unit glutathione-S-transferase.

REFERENCES:

Habig, W.H., Pabst, M.J., and Jakoby, W.B. (1974) *J. Biol. Chem.* **249**, 7130-7139.

NOTES:

1. This assay is based on 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.