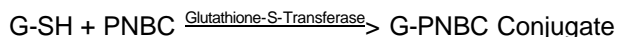


**Enzymatic Assay of GLUTATHIONE-S-TRANSFERASE  
(EC 2.5.1.18)  
p-Nitrobenzyl Chloride as Substrate**

**PRINCIPLE:**



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<sub>310nm</sub>, 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:

	<u>Test</u>	<u>Blank</u>
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	-----
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Immediately mix by inversion and record the increase in  $A_{310nm}$  for approximately 5 minutes. Obtain the  $r A_{310nm}/\text{minute}$  using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

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

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  $\mu\text{mole}$  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.**