



Product Information

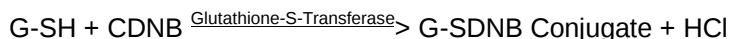
SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of GLUTATHIONE-S-TRANSFERASE

(EC 2.5.1.18)

1-Chloro-2,4-Dinitrobenzene as Substrate

PRINCIPLE:



Abbreviations:

G-SH = Glutathione, Reduced Form

CDNB = 1-Chloro-2,4-Dinitrobenzene

G-SDNB Conjugate = Glutathione-2,4-Dinitrobenzene

CONDITIONS: T = 25°C, pH = 6.5, A_{340nm}, 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, Sigma Prod. No. P-5379, and Ethylenediaminetetraacetic Acid, Tetrasodium Salt: Hydrate, Sigma Stock No. ED4S. Adjust to pH 6.5 at 25°C with 1 M KOH.)
- B. 75 mM Glutathione, Reduced Solution (G-SH)
(Prepare 10 ml in Reagent A using Glutathione, Free Acid, Reduced Form, Sigma Prod. No. G-4251. **PREPARE FRESH.**)
- C. 30 mM 1-Chloro-2,4-Dinitrobenzene Solution (CDNB)
(Prepare 1 ml in Reagent D using 1-Chloro-2,4-Dinitrobenzene, Sigma Prod. No. C-6396. **PREPARE FRESH.**)
- D. 95% Ethanol (Nondenatured)
- E. Glutathione-S-Transferase Enzyme Solution
(Immediately before use, prepare a solution containing 0.075 - 0.15 unit/ml of Glutathione-S-Transferase in cold Reagent A.)

**Enzymatic Assay of GLUTATHIONE-S-TRANSFERASE
(EC 2.5.1.18)
1-Chloro-2,4-Dinitrobenzene as Substrate**

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.70	2.80
Reagent B (G-SH)	0.10	0.10
Reagent C (CDNB)	0.10	0.10

Equilibrate to 25°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

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

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(3.0)(\text{df})}{(9.6)(0.10)}$$

3.0 = Total volume (in milliliters) of assay

df = Dilution factor

9.6 = Millimolar extinction coefficient of Glutathione-1-Chloro-2,4-Dinitrobenzene conjugate at 340 nm

0.10 = 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 1-chloro-2,4-dinitrobenzene with reduced glutathione per minute at pH 6.5 at 25°C.

**Enzymatic Assay of GLUTATHIONE-S-TRANSFERASE
(EC 2.5.1.18)
1-Chloro-2,4-Dinitrobenzene as Substrate**

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 97 mM potassium phosphate, 0.97 mM ethylenediaminetetraacetic acid, 2.5 mM glutathione, reduced, 1.0 mM 1-chloro-2,4-dinitrobenzene, 3.2% (v/v) ethanol and 0.0075 - 0.015 unit glutathione-S-transferase.

REFERENCES:

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

Simons, P.C., and Vander Jagt, D.L. (1977) *Anal. Biochem.* **82**, 334-341

NOTES:

1. This assay is based on the cited references.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

Sigma warrants that the above procedure information is currently utilized at Sigma and that all Sigma-Aldrich, Inc. products conform to the information in this and other Sigma-Aldrich, Inc. publications. Purchaser must determine the suitability of the information and product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.