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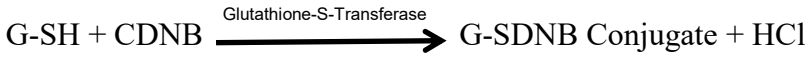
# 谷胱甘肽 S-转移酶

**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, A340nm, Light path = 1 cm

**METHOD：**

Continuous Spectrophotometric Rate Determination

**REAGENTS：**

A. 100 mM Potassium Phosphate Buffer with 1.0 mM Ethylenediaminetetraacetic Acid, pH 6.5at 25°C(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, and Ethylenediaminetetraacetic Acid, Tetrasodium Salt: Hydrate,Adjust to pH 6.5 at 25℃ with 1M KOH)

B. 75 mM Glutathione, Reduced Solution (G-SH)

(Prepare 10 ml in Reagent A using Glutathione, Free Acid, Reduced Form, PREPARE FRESH.)

C. 30 mM 1-Chloro-2,4-Dinitrobenzene Solution (CDNB)

(Prepare 1 ml in Reagent D using 1-Chloro-2,4-Dinitrobenzene, 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.)

**PROCEDURE:**

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

| | Test | Test |
|---|---|---|
| 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 A340nm until constant, using a suitably thermostatted spectrophotometer. Then add: | | |
| Reagent E (Enzyme Solution) | 0.10 | - |

Immediately mix by inversion and record the increase in A340nm for approximately 5 minutes. Obtain the ΔA340nm/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\Delta A340/\text{min Test} - \Delta A340/min \text{ Blank})(3.0)(df)}{(9.6)(0.10)}$$

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

$$\text{Units/mg solid} = \frac{units/ml\ enzyme}{mg\ solid/ml\ enzyme}$$

$$\text{Units/mg protein} = \frac{units/ml\ enzyme}{mg\ protein/ml\ enzyme}$$

**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.

**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.

**NOTES:**

1. This assay is based on the cited references.

2. Where Yuanye Product or Stock numbers are specified, equivalent reagents may be substituted.