

**Enzymatic Assay of THIOGLUCOSIDASE  
(EC 3.2.3.1)**

**PRINCIPLE:**

Thioglucoside + H<sub>2</sub>O  $\xrightarrow{\text{Thioglucosidase}}$  Thiol + Glucose

Glucose + ATP  $\xrightarrow{\text{Hexokinase}}$  Glucose 6-Phosphate + ADP

Glucose 6-Phosphate +  $\beta$ -NAD  $\xrightarrow{\text{G-6-P-DH}}$  6-Phosphogluconate +  $\beta$ -NADH

Abbreviations used:

ATP = Adenosine 5'-Triphosphate

ADP = Adenosine 5'-Diphosphate

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

G-6-P-DH = Glucose 6-Phosphate Dehydrogenase

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

**CONDITIONS:** T = 25°C, pH = 6.0, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

- A. 126 mM Sodium Phosphate Buffer with 37 mM Citric Acid and 1.0 mM Ascorbic Acid, pH 6.0 at 25°C  
(Prepare 1000 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous, Sigma Prod. No. S-0876, Citric Acid, Free Acid, Monohydrate, Sigma Prod. No. C-7129, and Ascorbic Acid, Sodium Salt, Sigma Prod. No. A-7631. Adjust to pH 6.0 at 25°C with either 1 M NaOH or 1 M HCl.)
- B. 4.8 mM Sinigrin Solution (Sinigrin)  
(Prepare 100 ml in Reagent A using Sinigrin, Monohydrate, Sigma Prod. No. S-1647.)
- C. Glucose Determination Kit (16-10)  
(Use Glucose (HK) Sigma Stock No. 16-10. Dissolve the contents of one 10 mg vial in 10 ml of deionized water.)
- D. Thioglucosidase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of Thioglucosidase in cold deionized water.)

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**PROCEDURE:**

Step 1:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Sinigrin)	9.00	9.00

Equilibrate to 25°C. Then add:

Reagent D (Enzyme Solution)	1.00	-----
Deionized Water	-----	1.00

Immediately mix by inversion and incubate for exactly 10 minutes.

Step 2:

Pipette (in milliliters) the following reagents into suitable cuvettes<sup>1</sup>:

Reagent C (16-10)	3.00	3.00
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Equilibrate to 25°C. Monitor the A<sub>340nm</sub> until constant using a suitably thermostatted spectrophotometer. Then add:

Test Solution (From Step 1)	0.10	-----
Blank Solution (From Step 1)	-----	0.10

Immediately mix by inversion and record the increase in A<sub>340nm</sub> for approximately 5 - 10 minutes until constant. Obtain the A<sub>340nm</sub> for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}} \text{ Test} - \Delta A_{340\text{nm}} \text{ Blank})(10)(3.1)(\text{df})}{(6.22)(1)(0.1)(10)}$$

10 = Total volume (in milliliters) of assay in Step 1

3.1 = Total volume (in milliliters) of assay in Step 2

df = Dilution factor

6.22 = Millimolar extinction coefficient of β-NADH at 340 nm

1 = Volume (in milliliters) of enzyme used in Step 1

0.1 = Volume (in milliliters) from Step 1 and Step 2

10 = Time of assay (in minutes) of Step 1 as per the Unit Definition

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**CALCULATIONS:** (continued)

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

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

**UNIT DEFINITION:**

One unit will produce 1.0  $\mu$ mole glucose per minute from sinigrin at pH 6.0 at 25°C.

**FINAL ASSAY CONCENTRATIONS:**

In a 10.00 ml reaction mix, the final concentrations are 113 mM sodium phosphate, 33 mM citric acid, 0.8 mM ascorbic acid, 4.3 mM sinigrin and 0.5 - 1.0 unit thioglucosidase.

**REFERENCES:**

Palmieri, S., Leoni, O., and Iori, R. (1982) *Analytical Biochemistry* **123**, 320-324.

Lenman, M., et al., (1990) *European Journal of Biochemistry* **194**, 747-753.

Wilkinson, A.P., Rhodes, M.J., and Fenwick, G.R. (1984) *Analytical Biochemistry* **139**, 284-291.

**NOTES:**

1. Step 2 should be done while the enzyme solution is incubating at 25°C in Step 1.
2. Hexokinase Unit Definition: One unit will phosphorylate 1.0  $\mu$ mole of D-glucose per minute at pH 7.6 at 25°C.
3. Glucose-6-Phosphate Dehydrogenase Unit Definition: One unit will oxidize 1.0  $\mu$ mole of D-glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of NADP or NAD at pH 7.8 at 30°C.
4. This assay is based on the cited references.

5. 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.**