Enzymatic Assay of α-GLUCOSIDASE
(EC 3.2.1.20)
p-Nitrophenyl α-D-Glucoside as Substrate
Product Nos. G5003, G6136, G7256, G8889, G0660, and G3651

PRINCIPLE:

p-Nitrophenyl α-D-Glucoside $\xrightarrow{\alpha\text{-Glucosidase}}$ α-D-Glucose + p-Nitrophenol

CONDITIONS: T = 37°C, pH = 6.8, A_{400nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 67 mM Potassium Phosphate Buffer, pH 6.8 at 37°C
(Prepare 100 mL in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No P5379. Adjust to pH 6.8 at 37°C with 1 M NaOH. **PREPARE FRESH.**)

B. 3 mM Glutathione, Reduced Solution (GSH)
(Prepare 10 mL in deionized water using L-Glutathione, Free Acid, Reduced Form, Sigma Prod. No. G4251. **PREPARE FRESH**.)

C. 10 mM p-nitrophenyl- α-D-Glucoside Solution (PNP-Gluc)
(Prepare 10 mL in deionized water using p-Nitrophenyl α-D-Glucopyranoside, Sigma Prod. No. N1377.)

D. 100 mM Sodium Carbonate Solution, (NaCarb)
(Prepare 50 mL in deionized water using Sodium Carbonate, Anhydrous, Sigma Prod. No. S2127.)

E. α-Glucosidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.15-0.3 unit/mL of α-Glucosidase in cold deionized water.)

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

	Test Solution	Blank Solution
Deionized Water	------	0.20
Reagent A (Buffer)	5.00	5.00
Reagent B (GSH)	0.20	0.20
Reagent E (Enzyme Solution)	0.20	------

Mix by inversion and equilibrate to 37°C.
Then add:

Reagent C (PNP Gluc)	0.50	0.50

Immediately mix by inversion and incubate for exactly 20 minutes at 37°C.

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

Test Solution	2.00	------
Blank Solution	------	2.00
Reagent D (NaCarb)	8.00	8.00

Mix by inversion and transfer the solutions to suitable cuvettes. Record the A_{400nm} for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{400nm}\ \text{Test} - A_{400nm}\ \text{Blank})(10)(5.9)(df)}{(18.3)(20)(2)(0.2)}$$

5.9 = Volume (in milliliters) of reaction mixture
df = Dilution factor
18.3 = Millimolar extinction coefficient of p-Nitrophenol at 400 nm
20 = Time (in minutes) of the assay
10 = Volume (in milliliters) of Colorimetric Determination
2 = Volume (in milliliters) of reaction mix used in the colorimetric determination

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 liberate 1.0 μmole of D-glucose from p-nitrophenyl α-D-glucoside per minute at pH 6.8 at 37°C.

FINAL ASSAY CONCENTRATIONS:
In a 5.90 mL reaction mix, the final concentrations are 57 mM potassium phosphate, 0.1 mM glutathione, 0.85 mM p-nitrophenyl α-D-glucoside and 0.03 – 0.06 units α-glucosidase.

NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.