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

**PRINCIPLE:**
p-Nitrophenyl $\alpha$-D-Glucoside $\xrightarrow{\alpha$-Glucosidase} $\alpha$-D-Glucose + p-Nitrophenol

**CONDITIONS:** $T = 37^\circ C$, $pH = 6.8$, $A_{400nm}$, 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- $\alpha$-D-Glucoside Solution (PNP-Gluc)
(Prepare 10 mL in deionized water using p-Nitrophenyl $\alpha$-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. $\alpha$-Glucosidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.15-0.3 unit/mL of $\alpha$-Glucosidase in cold deionized water.)
PROCEDURE:
Pipette (in milliliters) the following reagents into suitable containers:

<table>
<thead>
<tr>
<th></th>
<th>Test Solution</th>
<th>Blank Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Reagent B (GSH)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Reagent C (PNP Gluc)</th>
<th>0.50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.50</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th></th>
<th>Test Solution</th>
<th>Blank Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (NaCarb)</td>
<td>2.00</td>
<td>2.00</td>
</tr>
</tbody>
</table>

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:

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

5.9 = Volume (in milliliters) of reaction mixture
\(\text{df} = \) 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:

Units/mg solid = \( \frac{\text{units/mL enzyme}}{\text{mg solid/mL enzyme}} \)

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.