SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of GLUCOSE OXIDASE
(EC 1.1.3.4)

PRINCIPLE:

\[
\beta-D\text{-Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} D\text{-Glucono-1,5-Lactone} + H_2O_2
\]

\[
H_2O_2 + o\text{-Dianisidine (reduced)} \xrightarrow{\text{POD}} o\text{-Dianisidine (oxidized)}
\]

Abbreviations used:

GOD = Glucose Oxidase
POD = Peroxidase

CONDITIONS:  \( T = 35^\circ \text{C} \), \( pH = 5.1 \), \( A_{500\text{nm}} \), Light path = 1 cm

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A.  50 mM Sodium Acetate Buffer, pH 5.1 at 35°C
(Prepare 200 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.1 at 35°C with 1 M HCl.)

B.  0.21 mM o-Dianisidine Solution
(Dissolve the contents of one 50 mg vial of o-Dianisidine Dihydrochloride, Sigma Stock No. 510-50, in 7.6 ml of deionized water. Dilute 1.0 ml to 100 ml with Reagent A.)

C.  10% (w/v) \( \beta-D(+)\text{Glucose} \) Substrate Solution
(Prepare 10 ml in deionized water using \( \beta-D(+)\text{Glucose} \), Sigma Prod. No. G-5250.)

D.  0.17 mM o-Dianisidine and 1.72% (w/v) Glucose Solution (Reaction Cocktail)
(Immediately before use, prepare 29 ml by combining 24.0 ml of Reagent B with 5.0 ml of Reagent C. Equilibrate to 35°C and adjust to pH 5.1 if necessary with 1 M HCl or 1 M NaOH. PREPARE FRESH.)
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REAGENTS: (continued)

E. Peroxidase Enzyme Solution (POD)  
(Immediately before use, prepare a solution containing 60 Purpurogallin units/ml of Peroxidase, Type II, Sigma Prod. No. P-8250, in cold deionized water.)

F. Glucose Oxidase Enzyme Solution  
(For all Glucose Oxidase product numbers, except for crude products (Sigma Prod. Nos. G-6766 and G-1262) prepare an initial solution of 20 - 40 units/ml in cold Reagent A. Then immediately prior to use, further dilute to 0.4 - 0.8 unit in cold Reagent A. For crude products (Sigma Prod. Nos. G-6766 and G-1262), immediately prior to use prepare a solution of 0.4 - 2 units/ml in cold Reagent A.)¹

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>2.90</td>
<td>2.90</td>
</tr>
<tr>
<td>Reagent E (POD)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 35°C. Monitor the A₅₀₀nm until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in A₅₀₀nm for approximately 5 minutes. Obtain the ΔA₅₀₀nm/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

Units/ml enzyme = \[
\frac{(\Delta A_{500nm}/min \text{ Test} - \Delta A_{500nm}/min \text{ Blank}) \cdot (3.1) \cdot (df)}{(7.5) \cdot (0.1)}
\]

3.1 = Volume (in milliliters) of assay  
df = Dilution factor  
7.5 = Millimolar extinction coefficient of oxidized o-Dianisidine at 500 nm  
0.1 = Volume (in milliliters) of enzyme used
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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 oxidize 1.0 \( \mu \)mole of \( \beta \)-D-glucose to D-gluconolactone and \( \text{H}_{2}\text{O}_2 \) per minute at pH 5.1 at 35°C (equivalent to an \( \text{O}_2 \) uptake of 22.4 \( \mu \)l per minute). If the reaction mix is saturated with oxygen, the activity may increase by up to 100%.

FINAL ASSAY CONCENTRATION:

In a 3.10 ml reaction mix, the final concentrations are 48 mM sodium acetate, 0.16 mM o-dianisidine, 1.61% (w/v) glucose, and 6 units peroxidase (concentration will vary as to which glucose oxidase is used.)

REFERENCE:


NOTES:

1. a. Initial Enzyme Solutions: Prepare enzyme solutions in cold Reagent A in the concentrations indicated for the product numbers listed:
   Crude - Sigma Prod. Nos. G-1262 and G-6766, 0.2 mg solid/ml (no further dilutions are required)
   Type II - Sigma Prod. Nos. G-6125 and G-6641, 1.0 mg solid/ml Solution - Sigma Prod. Nos. G-6891 and G-9010, 0.1 ml solution and 5.00 ml Reagent A
   Type VII - Sigma Prod. Nos. G-2133 and G-7016 0.2 mg solid/ml
   Type X - Sigma Prod. No. G-7141, 0.2 mg solid/ml
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NOTES:  (continued)

1. Continued

   b. Final Dilutions: Immediately prior to use dilute the initial enzyme solutions to the following concentrations:
      Type II - 0.1 ml of 1.0 mg solid/ml and 5.00 ml of Reagent A
      Solutions - 0.1 ml of initial dilution and 3.00 ml of Reagent A
      Type VII - 0.1 ml of 0.2 mg solid/ml and 5.00 ml of Reagent A
      Type X - 0.1 ml of 0.2 mg solid/ml and 5.00 ml of Reagent A

2. Peroxidase Unit Definition: One POD unit will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20°C.

3. This assay is based on the cited reference.

4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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