Enzymatic Assay of GLUCONATE DEHYDROGENASE, NAD(P) Independent (EC 1.1.99.3)

**PRINCIPLE:**

\[ \text{D-Gluconate} + \text{DCIP} \xrightarrow{\text{GDH}} \text{2-Dehydro-D-Gluconate} + \text{Reduced DCIP} \]

Abbreviations used:
DCIP = 2,6-Dichlorophenol-Indophenol
GDH = Gluconate Dehydrogenase, NAD(P) Independent

**CONDITIONS:**  \( T = 25^\circ C, \ \text{pH} \ 6.0, \ \lambda_{600nm}, \ \text{Light path} = 1 \ \text{cm} \)

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 135 mM Potassium Phosphate Buffer, pH 6.0 at 25°C (Prepare 50 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Adjust to pH 6.0 at 25°C with 1 M KOH.)

B. 13 mM Phenazine Methosulfate Solution\(^1\) (PMS) (Prepare 1 ml in deionized water using Phenazine Methosulfate.)

C. 2.2 mM 2,6-Dichlorophenol-Indophenol Solution (DPIP) (Prepare 1 ml in deionized water using 2,6-Dichlorophenol-Indophenol, Sodium Salt)

D. 165 mM Sodium Gluconate Solution (Gluconate) (Prepare 2 ml in Reagent A using D-Gluconic Acid, Sodium Salt.)

E. 135 mM Potassium Phosphate Buffer with 0.05% (w/v) Bovine Serum Albumin, pH 6.0 at 25°C (Enz Dil) (Prepare 10 ml in Reagent A using Albumin, Bovine, Adjust to pH 6.0 at 25°C with either 1 M NaOH or 1 M HCl.)
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REAGENTS:  (continued)

F. Gluconate Dehydrogenase, NAD(P) Independent Enzyme Solution
(Immediately before use, prepare a solution containing 0.08 - 0.25 unit/ml of Gluconate Dehydrogenase, NAD(P) Independent in cold Reagent E.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent D (Gluconate)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent C (DPIP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent B (PMS)</td>
<td>0.10</td>
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</table>

Mix by inversion and equilibrate to 25°C. Monitor the A$_{600nm}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

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<thead>
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<tbody>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent E (Enz Dil)</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in A$_{600nm}$ for approximately 5 minutes. Obtain the $r$ A$_{600nm}$/minute using the maximum linear rate for both the Test and Blank.$^2$

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r_{A_{600nm}}} \text{ Test} - r_{A_{600nm}}} \text{ Blank}) \times l \times (df) \times 10 \times 0.1$$

1 = Total volume (in milliliter) of assay

df = Dilution factor

10 = Millimolar extinction coefficient of 2,6-dichlorophenol-indophenol at 600 nm

0.1 = Volume (in milliliter) of enzyme used

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

One unit will reduce 1.0 µmole of 2,6-dichlorophenol-indophenol per minute at 25°C at pH 6.0 in the presence of sodium gluconate.

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 108 mM potassium phosphate, 33 mM sodium gluconate, 0.22 mM 2,6-dichlorophenol-indophenol, 1.3 mM phenazine methosulfate, 0.005% (w/v) bovine serum albumin, and 0.008 – 0.025 unit gluconate dehydrogenase, NAD(P) independent.

REFERENCE:


NOTES:

1. Phenazine Methosulfate is used as an electron acceptor in the assay.

2. This enzyme exhibits a lag phase before the initial rate.

3. This assay is based on the cited reference.

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

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