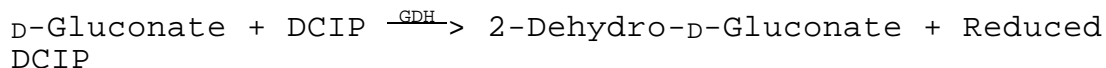


**Enzymatic Assay of GLUCONATE DEHYDROGENASE, NAD(P) Independent
(EC 1.1.99.3)**

PRINCIPLE:



Abbreviations used:

DCIP = 2,6-Dichlorophenol-Indophenol

GDH = Gluconate Dehydrogenase, NAD(P) Independent

CONDITIONS: T = 25°C, pH 6.0, A_{600nm}, Light path = 1 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, Sigma Prod. No. P-5379. Adjust to pH 6.0 at 25°C with 1 M KOH.)
- B. 13 mM Phenazine Methosulfate Solution¹ (PMS)
(Prepare 1 ml in deionized water using Phenazine Methosulfate, Sigma Prod. No. P-9625.)
- C. 2.2 mM 2,6-Dichlorophenol-Indophenol Solution (DPIP)
(Prepare 1 ml in deionized water using 2,6-Dichlorophenol-Indophenol, Sodium Salt, Sigma Prod. No. D-1878.)
- D. 165 mM Sodium Gluconate Solution (Gluconate)
(Prepare 2 ml in Reagent A using D-Gluconic Acid, Sodium Salt, Sigma Prod. No. G-9005.)
- 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, Sigma Prod. No. A-4503. Adjust to pH 6.0 at 25°C with either 1 M NaOH or 1 M HCl.)

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

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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.50	0.50
Reagent D (Gluconate)	0.20	0.20
Reagent C (DPIP)	0.10	0.10
Reagent B (PMS)	0.10	0.10

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

Reagent F (Enzyme Solution)	0.10	-----
Reagent E (Enz Dil)	-----	0.10

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:

$$\text{Units/ml enzyme} = \frac{(r A_{600nm} \text{ Test} - r A_{600nm} \text{ Blank})(1)(df)}{(10)(0.1)}$$

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

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

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

UNIT DEFINITION:

One unit will reduce 1.0 μ mole 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:

Matsushita, K., Shinagawa, E., Adachi, O., and Ameyama, M. (1979) *Journal of Biochemistry* **85**, 1173-1181

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 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.