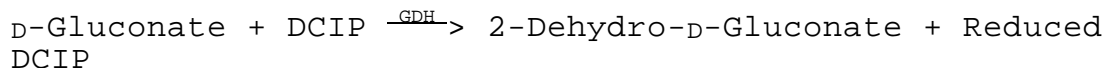


**Enzymatic Assay of GLUCONATE DEHYDROGENASE, NAD(P) Independent  
(EC 1.1.99.3)**

**PRINCIPLE:**



Abbreviations used:

DCIP = 2,6-Dichlorophenol-Indophenol

GDH = Gluconate Dehydrogenase, NAD(P) Independent

**CONDITIONS:** T = 25°C, pH 6.0,  $A_{600\text{nm}}$ , 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 Solution<sup>1</sup> (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.)

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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:

	<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.<sup>2</sup>

**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

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

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**UNIT DEFINITION:**

One unit will reduce 1.0  $\mu$ 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.**