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

PRINCIPLE:

$_D$-Gluconate + DCIP $\xrightarrow{GDH}$ 2-Dehydro-$_D$-Gluconate + Reduced DCIP

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 Solution[1] (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:

	Test	Blank
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

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

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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:

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.