

**Enzymatic Assay of GLUCONATE KINASE
(EC 2.7.1.12)**

PRINCIPLE:

D-Gluconate + ATP $\xrightarrow{\text{Gluconate Kinase}}$ 6-Phospho-D-Gluconate + ADP

6-Phospho-D-Gluconate + β -NADP $\xrightarrow[\text{Mg}^{2+}]{\text{G-PGDH}}$ D-Ribulose-5'-P + β -NADPH + CO₂

Abbreviations Used:

ATP = Adenosine 5'-Triphosphate

ADP = Adenosine 5'-Diphosphate

β -NADP = β -Nicotinamide Adenine Dinucleotide Phosphate,
Oxidized Form

G-PGDH = 6-Phosphogluconic Dehydrogenase

D-Ribulose-5'-P = D-Ribulose 5'-Phosphate

β -NADPH = β -Nicotinamide Adenine Dinucleotide Phosphate,
Reduced Form

CONDITIONS: T = 30°C, pH = 8.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Glycylglycine Buffer, pH 8.0 at 30°C
(Prepare 100 ml in deionized water using Gly-Gly, Free Base, Sigma Prod. No. G-1002. Adjust to pH 8.0 at 30°C with 1 M NaOH.)
- B. 100 mM Magnesium Chloride Solution (MgCl₂)
(Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
- C. 110 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 10 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-6144. Neutralize with Sodium Bicarbonate, Sigma Prod. No. S-8875 until the effervescence ceases.)

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REAGENTS: (continued)

- D. 13 mM β -Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form, Solution (β -NADP)
(Prepare 5 ml in deionized water using β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505.)
- E. 92 mM D-Gluconic Acid Solution (Gluconic Acid)
(Prepare 5 ml in deionized water using D-Gluconic Acid, Sodium Salt, Sigma Prod. No. G-9005.)
- F. 6-Phosphogluconic Dehydrogenase Enzyme Solution (6-PGDH)
(Immediately before use, prepare a solution containing 50 units/ml of 6-Phosphogluconic Dehydrogenase, Sigma Prod. No. P-0632, in cold deionized water.)
- G. Gluconate Kinase Enzyme Solution (GK)
(Immediately before use, prepare a solution containing 0.2 - 0.4 unit/ml of Gluconate Kinase in cold deionized water.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Reagent A (Buffer)	24.00
Reagent B ($MgCl_2$)	1.00
Reagent C (ATP)	1.00
Reagent D (β -NADP)	1.00
Reagent E (Gluconic Acid)	1.00

Mix by swirling and equilibrate to 30°C. Adjust to pH 8.0 with either 1 M NaOH or 1 M HCl.

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.80	2.80
Reagent F (6-PGDH)	0.10	0.10

Mix by inversion and equilibrate to 30°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent G (GK)	0.10	-----
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Deionized Water

0.10

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PROCEDURE: (continued)

Immediately mix by inversion and record the increase in $A_{340\text{nm}}$ for approximately 15 minutes. Obtain the $r A_{340\text{nm}}$ /minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3)(\text{df})}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β -NADPH at 340 nm

0.1 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will convert 1.0 μ mole of D-gluconate to 6-phospho-D-gluconate per minute at pH 8.0 at 30°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mixture, the final concentrations are 80 mM glycylglycine, 3.3 mM magnesium chloride, 3.7 mM adenosine 5'-triphosphate, 0.43 mM β -nicotinamide adenine dinucleotide phosphate, 3.1 mM D-gluconic acid, 5 units 6-phosphogluconic dehydrogenase, and 0.02 - 0.04 unit gluconate kinase.

REFERENCE:

Leder, I.G. (1957) *Journal of Biological Chemistry*, 225, 125-136

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NOTES:

1. 6-Phosphogluconic Dehydrogenase Unit Definition: One unit will oxidize 1.0 μ mole of 6-phospho-D-gluconate to D-ribulose 5'-phosphate and CO₂ per minute at pH 7.4 at 37°C in the presence of NADP.
2. This assay is based on the cited reference.
3. 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.