Enzymatic Assay of 6-PHOSPHOGLUCONIC DEHYDROGENASE[1] (EC 1.1.1.44)

PRINCIPLE:

6-PGluc + ß-NADP $\xrightarrow{\text{6-PGDH}}$ D-Ribulose 5'-Phosphate + CO_2 + NADPH

Abbreviations used:
6-PGluc = 6-Phosphogluconate
6-PGDH = 6-Phosphogluconic Dehydrogenase
ß-NADP = ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
ß-NADPH = ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

CONDITIONS: T = 37°C, pH 7.4, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 250 mM Glycylglycine Buffer, pH 7.4 at 37°C.
(Prepare 50 ml in deionized water using Glycylglycine, Hydrochloride, Prod. No. G-1127. Adjust to pH 7.4 at 37°C with 1 M NaOH.)

B. 60 mM 6-Phosphogluconate Solution (6-PGluc)
(Prepare 1 ml in deionized water using 6-Phosphogluconic Acid, Trisodium Salt, Prod. P-7877.)

C. 20 mM ß-Nicotinamide Adenine Dinucleotide Phosphate Solution (ß-NADP)
(Prepare 2 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Prod No. N-0505. **PREPARE FRESH**.)

D. 300 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 1 ml in deionized water using Magnesium Chloride Hexahydrate, Prod. No. M-0250.)

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REAGENTS: (continued)

E. 6-Phosphogluconic Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.3 - 0.5 unit/ml of 6-Phosphogluconic Dehydrogenase in cold deionized water.)

PROCEDURE:

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

	Test	Blank
Deionized Water	1.98	2.08
Reagent A (Buffer)	0.62	0.62
Reagent B (6-PGlu)	0.10	0.10
Reagent C (ß-NADP)	0.10	0.10
Reagent D ($MgCl_2$)	0.10	0.10

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent E (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the increase in A_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{\Delta A_{340nm}/\text{min Test} - A_{340nm}/\text{min Blank}}{(6.22)\ (\text{mg enzyme/ml RM})}$$

6.22 = Millimolar extinction coefficient of ß-NADPH at 340 nm
RM = Reaction Mix

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UNIT DEFINITION:

One unit will oxidize 1.0 µmole of 6-phospho-D-gluconate to D-ribulose 5-phosphate and CO_2 per minute at pH 7.4 at 37°C in the presence of ß-NADP.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 52 mM glycylglycine, 2.0 mM 6-phosphogluconate, 0.67 mM ß-NADP, 10 mM magnesium chloride and 0.03 - 0.05 unit 6-phosphogluconic dehydrogenase.

REFERENCE:

Bergmeyer, H.U. (1974) *Methods of Enzymatic Analysis*, Second Edition, Volume I, 500-501.

NOTES:

1. Not to be used with 6-Phosphogluconic Dehydrogenase, from Leuconostoc mesenteroides, Prod. No. P-7281.

2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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