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

PRINCIPLE:

6-PGluc + ß-NAD $\xrightarrow{\text{6-PGDH}}$ $_D$-Ribulose 5'-Phosphate + CO_2 + NADH

Abbreviations used:
6-PGluc = 6-Phosphogluconate
6-PGDH = 6-Phosphogluconic Dehydrogenase
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 25°C, pH 7.5, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Glycylglycine Buffer, pH 7.5 at 25°C.
(Prepare 50 ml in deionized water using Glycylglycine, Free Base, Sigma Prod. No. G-1002. Adjust to pH 7.5 at 25°C with 1 M HCl.)

B. 100 mM 6-Phosphogluconate Solution (6-PGluc)
(Prepare 1 ml in deionized water using 6-Phosphogluconic Acid, Trisodium Salt, Sigma Prod. P-7877.)

C. 60 mM ß-Nicotinamide Adenine Dinucleotide Solution (ß-NAD)
(Prepare 1 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004. **PREPARE FRESH.**)

D. 6-Phosphogluconic Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 1.5 - 3.0 units/ml of 6-Phosphogluconic Dehydrogenase in cold deionized water.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.75	2.77
Reagent B (6-PGlu)	0.05	0.05
Reagent C (ß-NAD)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution)	0.02

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

CALCULATIONS:

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

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

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of 6-phospho-$_{D}$-gluconate to $_{D}$-ribulose 5-phosphate and CO_2 per minute to pH 7.5 at 25°C in the presence of ß-NAD.

FINAL ASSAY CONCENTRATIONS:

In a 2.92 ml reaction mix, the final concentrations are 94 mM glycylglycine, 1.7 mM 6-phosphogluconate, 2.0 mM ß-NAD and 0.03 - 0.06 unit 6-phosphogluconic dehydrogenase.

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NOTES:

1. This assay is not to be used to assay enzyme activity of Sigma Prod. No.'s P-4553, P-0507, P-0632, P-7533, and P-8406.

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.