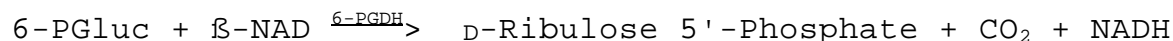


**Enzymatic Assay of 6-PHOSPHOGLUCONIC DEHYDROGENASE<sup>1</sup>**  
**(EC 1.1.1.44)**

**PRINCIPLE:**



Abbreviations used:

6-PGluc = 6-Phosphogluconate

6-PGDH = 6-Phosphogluconic Dehydrogenase

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide,  
Reduced Form

**CONDITIONS:** T = 25°C, pH 7.5, A<sub>340nm</sub>, 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  $\beta$ -Nicotinamide Adenine Dinucleotide Solution ( $\beta$ -NAD)  
(Prepare 1 ml in deionized water using  $\beta$ -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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.75	2.77
Reagent B (6-PGlu)	0.05	0.05
Reagent C (β-NAD)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the A<sub>340nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution)		0.02 -----
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Immediately mix by inversion and record the increase in A<sub>340nm</sub> for approximately 5 minutes. Obtain the r A<sub>340nm</sub>/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

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

6.22 = Millimolar extinction coefficient of β-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<sub>2</sub> per minute to pH 7.5 at 25°C in the presence of β-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 β-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.**