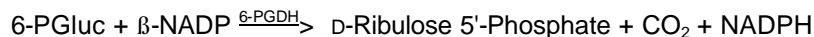


## Enzymatic Assay of 6-PHOSPHOGLUCONIC DEHYDROGENASE<sup>1</sup> (EC 1.1.1.44)

### PRINCIPLE:



Abbreviations used:

6-PGLuc = 6-Phosphogluconate

6-PGDH = 6-Phosphogluconic Dehydrogenase

$\beta$ -NADP =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Oxidized Form

$\beta$ -NADPH =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Reduced Form

**CONDITIONS:** T = 37°C, pH 7.4,  $A_{340\text{nm}}$ , 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  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate Solution ( $\beta$ -NADP)  
(Prepare 2 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Sodium Salt, Prod No. N-0505. **PREPARE FRESH.**)
- D. 300 mM Magnesium Chloride Solution ( $\text{MgCl}_2$ )  
(Prepare 1 ml in deionized water using Magnesium Chloride Hexahydrate, Prod.  
No. M-0250.)

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

**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:

	<u>Test</u>	<u>Blank</u>
Deionized Water	1.98	2.08
Reagent A (Buffer)	0.62	0.62
Reagent B (6-PGlu)	0.10	0.10
Reagent C (β-NADP)	0.10	0.10
Reagent D (MgCl <sub>2</sub> )	0.10	0.10

Mix by inversion and equilibrate to 37°C. Monitor the A<sub>340nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

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

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

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

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

### UNIT DEFINITION:

One unit will oxidize 1.0  $\mu$ mole of 6-phospho-D-gluconate to D-ribulose 5-phosphate and CO<sub>2</sub> per minute at pH 7.4 at 37°C in the presence of  $\beta$ -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  $\beta$ -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.**