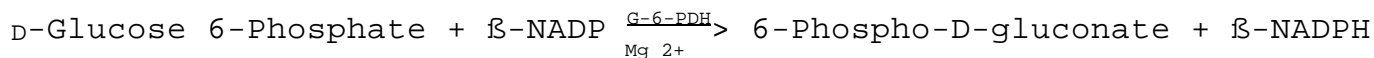


**Enzymatic Assay of GLUCOSE-6-PHOSPHATE DEHYDROGENASE
(EC 1.1.1.49)**

PRINCIPLE:



Abbreviations used:

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

G-6-PDH = Glucose-6-Phosphate Dehydrogenase

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

CONDITIONS: T = 30°C, pH = 9.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Tris HCl Buffer, pH 9.0 at 30°C
(Prepare 100 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253. Adjust to pH 9.0 at 30°C with 1 M NaOH.)
- B. 100 mM D-Glucose 6-Phosphate Solution (G 6-P)
(Prepare 2 ml in deionized water using D-Glucose 6-Phosphate, Monopotassium Salt, Sigma Prod. No. G-6526.)
- C. 20 mM β -Nicotinamide Adenine Dinucleotide Phosphate Solution (β -NADP)
(Dissolve the contents of one 10 mg vial of β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Stock. No. 240-310, in the appropriate volume of deionized water.)
- D. 600 mM Magnesium Chloride Solution (MgCl₂)
(Prepare 2 ml in deionized water using Magnesium Chloride Hexahydrate, Sigma Prod. No. M-0250.)

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REAGENTS: (continued)

E. Glucose-6-Phosphate Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing
0.3 - 0.6 unit/ml of Glucose-6-Phosphate Dehydrogenase
in cold deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.52	2.52
Reagent B (G 6-P)	0.08	0.08
Reagent C (β-NADP)	0.10	0.10
Reagent D (MgCl ₂)	0.20	0.20

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

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

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.

CALCULATION:

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

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 milliliters) of enzyme used

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

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CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will oxidize 1.0 μ mole of D-glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of β -NADP at pH 9.0 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 84 mM Tris, 2.7 mM D-glucose 6-phosphate, 0.67 mM β -nicotinamide adenine dinucleotide phosphate, 40 mM magnesium chloride, and 0.03 - 0.06 unit glucose-6-phosphate dehydrogenase.

NOTE:

1. 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.