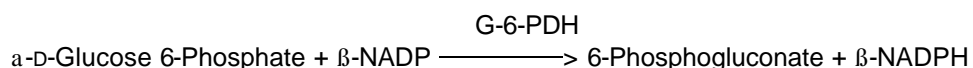
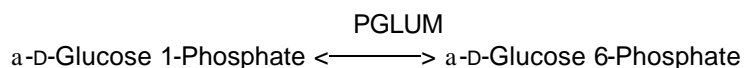


Enzymatic Assay of PHOSPHORYLASE a (EC 2.4.1.1)

PRINCIPLE:



Abbreviations used:

P_i = Inorganic Phosphate

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

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

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

PGLUM = Phosphoglucomutase

CONDITIONS: T = 30°C, pH = 6.8, $A_{340\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 500 mM Potassium Phosphate Buffer, pH 6.8 at 30°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.8 at 30°C with 1 M KOH.)
- B. 4% (w/v) Glycogen Solution (Glycogen)
(Prepare 10 ml in deionized water using Glycogen, Sigma Prod. No. G-8876.)
- C. 300 mM Magnesium Chloride Solution (MgCl_2)
(Prepare 5 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
- D. 100 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 2 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium, Hydrate, Sigma Stock No. ED4SS.)

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REAGENTS: (continued)

- E. 6.5 mM β -Nicotinamide Adenine Dinucleotide Phosphate Solution (β -NADP)
(Prepare 15 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, Phosphate, Sodium, Sigma Prod. No. N-0505. **PREPARE FRESH.**)
- F. 0.1% (w/v) α -D-Glucose 1,6-Diphosphate Solution (G 1,6-DiP)
(Prepare 1 ml in deionized water using α -D-Glucose 1,6-Diphosphate, Cyclohexylammonium Salt, Hydrate, Sigma Prod. No. G-5875.)
- G. Glucose-6-Phosphate Dehydrogenase Solution (G-6-PDH)
(Immediately before use, prepare a solution containing 10 units/ml in cold deionized water using Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. G-6378.)
- H. Phosphoglucomutase Solution (PGLUM)
(Immediately before use, prepare a solution containing 10 units/ml in cold deionized water using Phosphoglucomutase, Sigma Prod. No. P-3397.)
- I. 40 mM β -Glycerophosphate with 80 mM Cysteine Solution, pH 6.8 at 30°C (Diluent)
(Prepare 20 ml in deionized water using β -Glycero-phosphate, Disodium Salt, Hydrate, Sigma Prod. No. G-6251, and L-Cysteine, Hydrochloride, Monohydrate, Sigma Prod. No. C-7880. Adjust to pH 6.8 with 1 M NaOH.)
- J. Phosphorylase a Enzyme Solution
(Immediately before use, prepare a solution containing 0.25 unit/ml of Phosphorylase a in cold Reagent I.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Deionized water		99.50
Reagent A (Buffer)		15.00
Reagent B (Glycogen)		7.50
Reagent C ($MgCl_2$)		0.67
Reagent D (EDTA)	0.15	
Reagent E (β -NADP)		10.00
Reagent F (G 1,6-DiP)		0.50

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PROCEDURE: (continued)

Mix and adjust to pH 6.8 at 30°C with 100 mM HCl or 100 mM NaOH, if necessary.

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.70	2.70
Reagent G (G-6-PDH)	0.10	0.10
Reagent H (PGLUM)	0.10	0.10

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

Reagent I (Diluent)	-----	0.10
Reagent J (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the increase in $A_{340\text{nm}}$ for approximately 10 minutes. Obtain the $r A_{340\text{nm}}/\text{minute}$ using the maximum linear rate¹ for both the Test and Blank.

CALCULATIONS:

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

3 = Total volume (in milliliters) of the assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β -NADH at 340 nm

0.1 = Volume (in milliliter) of enzyme used

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

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

Enzymatic Assay of PHOSPHORYLASE a (EC 2.4.1.1)

UNIT DEFINITION:

One unit will form 1.0 μ mole of α -D-glucose 1-phosphate from glycogen and orthophosphate per minute at pH 6.8 at 30°C, measured in a system containing phosphoglucomutase, β -NADP, and glucose-6-phosphate dehydrogenase.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 51 mM potassium phosphate, 0.20% (w/v) glycogen, 1.4 mM magnesium chloride, 0.10 mM ethylenediaminetetraacetic acid, 0.44 mM β -Nicotinamide Adenine Dinucleotide Phosphate, Oxidized form, 0.0003% (w/v) α -D-glucose 1,6-diphosphate, 1.3 mM β -glycerophosphate, 2.7 mM cysteine, 1 unit glucose-6-phosphate dehydrogenase, 1 unit phosphoglucomutase, and 0.025 unit phosphorylase a.

NOTES:

1. The maximum linear rate should not exceed a ΔA_{340nm} /minute of 0.1.
2. Glucose-6-Phosphate Dehydrogenase 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 7.4 at 25°C.
3. Phosphoglucomutase Unit Definition: One unit will convert 1.0 μ mole of α -D-glucose 1-phosphate to α -D-glucose 6-phosphate per minute at pH 7.4 at 30°C.
4. 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.