**Enzymatic Assay of PHOSPHORYLASE a**
**(EC 2.4.1.1)**

**PRINCIPLE:**

$$\text{Glycogen}_n + P_i \xrightarrow{\text{Phosphorylase a}} \text{Glycogen}_{n-1} + \text{a-D-Glucose 1-Phosphate}$$

$$\text{a-D-Glucose 1-Phosphate} \xleftrightarrow{\text{PGLUM}} \text{a-D-Glucose 6-Phosphate}$$

$$\text{a-D-Glucose 6-Phosphate} + \text{ß-NADP} \xrightarrow{\text{G-6-PDH}} \text{6-Phosphogluconate} + \text{ß-NADPH}$$

Abbreviations used:
$P_i$ = Inorganic Phosphate
ß-NADP = ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
ß-NADH = ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
G-6-PDH = Glucose-6-Phosphate Dehydrogenase
PGLUM = Phosphoglucomutase

**CONDITIONS:** T = 30°C, pH = 6.8, $A_{340nm}$, 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 ß-Nicotinamide Adenine Dinucleotide Phosphate Solution (ß-NADP)
(Prepare 15 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Phosphate, Sodium, Sigma Prod. No. N-0505. **PREPARE FRESH.**)

F. 0.1% (w/v) a-D-Glucose 1,6-Diphosphate Solution (G 1,6-DiP)
(Prepare 1 ml in deionized water using a-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 ß-Glycerophosphate with 80 mM Cysteine Solution, pH 6.8 at 30°C (Diluent)
(Prepare 20 ml in deionized water using ß-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 (ß-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:

| | Test | Blank |
|---|---|---|
| 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 $A_{340nm}$ 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 $A_{340nm}$ for approximately 10 minutes. Obtain the $\Delta A_{340nm}$/minute using the maximum linear rate[1] for both the Test and Blank.

**CALCULATIONS:**

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

3 = Total volume (in milliliters) of the assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.1 = Volume (in milliliter) of enzyme used

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

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

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**UNIT DEFINITION:**

One unit will form 1.0 µmole of a-D-glucose 1-phosphate from glycogen and orthophosphate per minute at pH 6.8 at 30°C, measured in a system containing phosphoglucomutase, ß-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 ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized form, 0.0003% (w/v) a-D-glucose 1,6-diphosphate, 1.3 mM ß-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 ?$A_{340nm}$/minute of 0.1.

2. Glucose-6-Phosphate Dehydrogenase Unit Definition: One unit will oxidize 1.0 µmole of D-glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of ß-NADP at pH 7.4 at 25°C.

3. Phosphoglucomutase Unit Definition: One unit will convert 1.0 µmole of a-D-glucose 1-phosphate to a-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.**