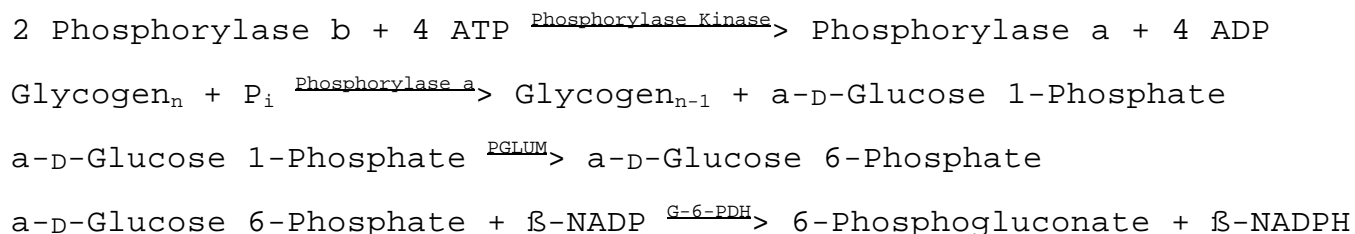


**Enzymatic Assay of PHOSPHORYLASE KINASE
(EC 2.7.1.38)**

PRINCIPLE:



Abbreviations used:

ATP = Adenosine 5'-Triphosphate

ADP = Adenosine 5'-Diphosphate

P_i = Inorganic Phosphate

PGLUM = Phosphoglucomutase

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 = 7.7, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 30 mM L-Cysteine Solution, pH 7.0 at 30°C (L-Cys, pH 7.0)
(Prepare 50 ml in deionized water using L-Cysteine, Hydrochloride, Monohydrate, Sigma Prod. No. C-7880. Adjust to pH 7.0 at 30°C with solid Sodium Bicarbonate, Sigma Prod. No. S-8875. **PREPARE FRESH.**)
- B. 80 mM L-Cysteine Solution, pH 6.8 at 30°C (L-Cys, pH 6.8)
(Prepare 50 ml in deionized water using L-Cysteine, Hydrochloride, Monohydrate, Sigma Prod. No. C-7880. Adjust to pH 6.8 at 30°C with solid Sodium Bicarbonate, Sigma Prod. No. S-8875. **PREPARE FRESH.**)
- C. 250 mM Tris HCl Buffer, pH 7.7 at 30°C
(Prepare 100 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253. Adjust to pH 7.7 at 30°C with 1 M NaOH.)

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REAGENTS: (continued)

- D. 250 mM β -Glycerophosphate¹ Solution (β -Glycerophos)
(Prepare 10 ml in Reagent C using β -Glycerophosphate, Disodium Salt, Hydrate, Sigma Prod. No. G-6251.)
- E. 40 mM β -Glycerophosphate Solution, pH 6.8 at 30°C
(β -Glycerophos, pH 6.8)
(Prepare 50 ml in Reagent B using β -Glycerophosphate, Disodium Salt, Hydrate, Sigma Prod. No. G-6251. Adjust to pH 6.8 at 30°C with 1 M HCl or 1 M NaOH, if necessary.)
- F. 100 mM Magnesium Acetate Solution, pH 7.7 at 30°C
(Mg Acet)
(Prepare 10 ml in deionized water using Magnesium Acetate, Tetrahydrate, Sigma Prod. No. M-9147. Adjust to pH 7.7 at 30°C with 1 M NaOH.)
- G. Phosphorylase b Enzyme Solution (Phos b)
(Immediately before use, prepare a solution containing approximately 300 units/ml of Phosphorylase b in cold Reagent A.)
- H. 30 mM Adenosine 5'-Triphosphate Solution, pH 7.7 at 30°C (ATP)
(Prepare 10 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394. Adjust to pH 7.7 at 30°C with 1 M NaOH.)
- I. Phosphorylase Kinase Enzyme Solution (Phos Kinase)
(Immediately before use, prepare a solution containing approximately 75 units/ml of Phosphorylase Kinase in cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Reagent A (L-Cys, pH 7.0)	0.29	0.30
Reagent D (β -Glycerophos)	0.25	0.25
Reagent F (Mg Acet)	0.10	0.10
Reagent G (Phos b)	0.25	0.25
Reagent I (Phos Kinase)		0.01

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 30°C. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent H (ATP)	0.10	0.10

Immediately mix by inversion and incubate at 30°C. After exactly 5 and 10 minutes, remove 0.1 ml from both the Test and Blank solutions and place into suitable tubes containing 1.90 ml of Reagent E (β-Glycerophos, pH 6.8) to stop the reaction.

Assay for Phosphorylase a activity according to the attached procedure (Enzymatic Assay of Phosphorylase a), substituting 0.10 ml from each of the aforementioned stopped reactions in place of Reagent J (Phosphorylase a Enzyme Solution) in the attached assay. Proceed with the calculation.

CALCULATIONS:

Phosphorylase a Activity (see attached procedure):

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.1 = Volume (in milliliter) of enzyme used

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

Phosphorylase Kinase Activity:

Units/ml enzyme =

$$\frac{(\text{units of Phos a Test/ml}) - (\text{units of Phos a Blank/ml})}{(T)(0.01)(0.1)} \quad (2)$$

2 = Volume (in milliliters) of the stopped reaction (Phosphorylase Kinase Assay)

T = Time (in minutes) of the assay (Phosphorylase Kinase Assay) as per the Unit Definition

0.1 = Volume (in milliliter) of reaction mix used in the stopped reaction

0.01 = Volume (in milliliter) of enzyme used in

Phosphorylase
Kinase assay

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CALCULATIONS: (continued)

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

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

UNIT DEFINITION:

One unit will form 1.0 μ molar unit of phosphorylase a from phosphorylase b per minute at pH 7.7 at 30°C in the presence of ATP.

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 17 mM L-cysteine, 63 mM β -glycerophosphate, 10 mM magnesium acetate, 75 units phosphorylase b, 63 mM Tris, and 0.75 unit phosphorylase kinase.

REFERENCES:

Krebs, E.G. (1966) *Methods in Enzymology*, VIII, 543-546

NOTES:

1. β -Glycerophosphate is a stabilizer for phosphorylase kinase.
2. This assay is based on the cited reference.
3. Phosphorylase b Unit Definition: One unit will form 1.0 μ mole of α -D-glucose 1-phosphate from glycogen and orthophosphate in the presence of 5'-AMP, per minute at pH 6.8 at 30°C measured in a system containing phosphoglucomutase, β -NADP, and glucose 6-phosphate dehydrogenase.
4. 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.