

Phosphorylase-b, Enzyme Activity

Catalog	Unit
TBP0084-10MG	10 mg
TBP0084-50MG	50 mg

Product Details

Form: Freeze-dried

Solubility: Soluble in distilled water or dilute buffer

Stability: -20° C; -4° F

Activity: 60 U/mg protein

Protein: 90%

Unit Definition

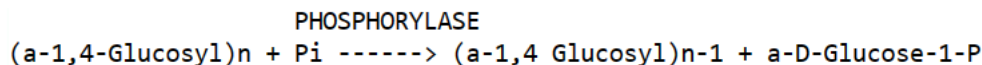
That amount of enzyme which will liberate one micromole of glucose-1-phosphate from glycogen and orthophosphate, in the presence of 5'-AMP, per minute at pH 6.8 at 37°C.

Assay Method

The assay method which is described.

Applications

Phosphorylases (EC 2.4.1.1.) catalyze the breakdown of glycogen in liver and muscle. Similar enzymes, occurring in plants, catalyze the hydrolysis of starch. The hydrolysis of glycogen is represented by the following reaction:



The phosphorylase of skeletal muscle occurs in two forms, the active form (phosphorylase-a) and a much less active form (phosphorylase-b). These two forms are interconvertible. Rabbit muscle phosphorylase has been extensively studied. Phosphorylase-a has a molecular weight of 370,000 and phosphorylase-b has a molecular weight of 185,000.

Reagents

- 0.05 M Potassium phosphate buffer, pH 6.8 containing 0.1 mM EDTA.
- 0.1 M Magnesium chloride in distilled water.
- NADP (10 mg/ml) in distilled water. Prepare fresh.
- Glycogen (6 mg/ml) in buffer. Prepare fresh.
- 1 mM adenosine 5'-monophosphate (sodium salt) in buffer. Prepare fresh.
- Phosphoglucomutase (PGM) solution. Prepare a solution in cold 1% bovine serum albumin to yield a concentration of 20 U/ml. Must be prepared fresh.
- Glucose-6-P-dehydrogenase (G6P-DH) solution. Prepare a solution in cold 1% bovine serum albumin to yield a final concentration of 100 U/ml. Must be prepared fresh.
- Phosphorylase (enzyme) solution. Prepare a solution in cold 1% bovine serum albumin to yield a final concentration of 2-4 U/ml. Must be prepared fresh immediately prior to assay. (Note: the enzyme preparation contains 5'-AMP which is needed for its catalytic activity.)

Calculation

$$\text{Activity (U/mg)} = \frac{(\Delta E_{340\text{nm}/\text{min}})(\text{Total Vol.})(\text{Enz. Diln.})}{(6.22)(\text{Enz. Vol.})(\text{mg Enz./ml})}$$

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