上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：400-666-5481　传真：021-55068248
网址：www.shyuanye.com
邮箱：3008007435@qq.com

# 3-Phosphoglyceric Phosphokinase

**PRINCIPLE：**

GAP+β-NAD+$P_i$→（GAPDH）→1,3-Diphosphoglycerate + β-NADH

1,3-Diphosphoglycerate + ADp→（3PGK）→ 3-Phosphoglycerate + ATP

Abbreviations used:

GAP=Glyceraldehyde 3-Phosphate

β-NAD= β-Nicotinamide Adenine Dinucleotide, Oxidized Form

Pi= lnorganic Phosphate

GAPDH=Glyceraldehyde-3-Phosphate Dehydrogenase

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

ADP = Adenosine 5-Diphosphate

3-PGK=3-Phosphoglyceric Phosphokinase

4-ATP = Adenosine 5'-Triphosphate

**CONDITIONS：**

T=25℃, pH 6.9, A34onm, Light path=1 cm

**METHOD：**

Continuous Spectrophotometric Rate Determination

**REAGENTS：**

A.100 mM Potassium Phosphate Buffer, pH 6.9 at 25℃

(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous. Adjust to pH 6.9 at 25℃ with 1 M KOH.)

B.25 mM DL-Glyceraldehyde-3-Phosphate Solution (GAP)

(Prepare 1 ml in deionized water using DL-Glyceraldehyde 3-Phosphate, Free Acid.)

C.20 mM B-Nicotinamide Adenine Dinucleotide, Oxidized Form Solution ( β -NAD)

(Prepare 1 ml in deionized water using β -Nicotinamide Adenine Dinucleotide.)

D.10 mM Adenosine 5'-Diphosphate Solution (ADP)

(Prepare 1 ml in deionized water using Adenosine 5-Diphosphate, Sodium Salt. Prepare Fresh.)

E. 50 mM Magnesium Sulfate Solution (MgSO4)

(Prepare 10 ml in deionized water using Magnesium Sulfate, Heptahydrate.)

F. 400 mM Glycine Solution (Glycine)

(Prepare 25 ml in deionized water using Glycine, Free Base.)

G. Glyceraldehyde-3-Phosphate Dehydrogenase Solution (GAPDH)

(Immediately before use, prepare a solution containing 20 units/ml of Glyceraldehyde 3-Phosphate Dehydrogenase in cold deionized water.)

H. 3-Phosphoglyceric Phosphokinase Enzyme Solution (3-PGK)

(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of 3-Phosphoglyceric Phosphokinase in cold Reagent A.)

**PROCEDURE:**

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

| | Test | Blank |
|---|---|---|
| Reagent A (Buffer) | 1.40 | 1.40 |
| Reagent B (GAP) | 0.10 | 0.10 |
| Reagent C(B-NAD) | 0.05 | 0.05 |
| Reagent D(ADP) | 0.05 | 0.05 |
| Reagent E(MgSO) | 0.25 | 0.25 |
| Reagent F(Glycine) | 1.00 | 1.00 |
| Reagent G(GAPDH) | 0.05 | 0.05 |
| Mix by inversion and equilibrate to 25.C. Monitor the As4onm until constant, using a suitablythermostatted spectrophotometer. Then add: | | |
| Reagent H (3-PGK) | 0.10 | —— |
| Reagent A (Buffer) | —— | 0.10 |

Immediately mix by inversion and record the increase in As4onm for approximately 5 minutes.Obtain the AA34onm/min using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\Delta A34onm/min\ Test - \Delta A34onm/min\ Blank)(3)(df)}{(6.22)(0.1)}$$

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

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

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

**UNIT DEFINITION:**

One unit will convert 1.0 umole of 1,3-diphosphoglycerate to 3-phosphoglycerate per minute at pH6.9 at 25℃.

**FINAL ASSAY CONCENTRATIONS:**

In a 3.00 ml reaction mix, the final concentrations are 50 mM potassium phosphate,0.83 mM glyceraldehyde-3-phosphate, 0.3mM β-nicotinamide adenine dinucleotide,0.2 mM adenosine 5′-diphosphate, 4.2 mM magnesium sulfate, 133 mM glycine,1 unit glyceraldehyde-3-phosphate dehydrogenase, and 0.03 - 0.06 unit 3-phosphoglyceric phosphokinase.