SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of 3-PHOSPHOGLYCERIC PHOSPHOKINASE
(EC 2.7.2.3)
from Baker's Yeast

PRINCIPLE:

GAP + β-NAD + P_i $\xrightarrow{\text{GAPDH}}$ 1,3-Diphosphoglycerate + β-NADH

1,3-Diphosphoglycerate + ADP $\xrightarrow{\text{3-PGK}}$ 3-Phosphoglycerate + ATP

Abbreviations used:
GAP = Glyceraldehyde 3-Phosphate
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form
P_i = Inorganic Phosphate
GAPDH = Glyceraldehyde-3-Phosphate Dehydrogenase
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form
ADP = Adenosine 5'-Diphosphate
3-PGK = 3-Phosphoglyceric Phosphokinase
ATP = Adenosine 5'-Triphosphate

CONDITIONS: T = 25°C, pH 6.9, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 6.9 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.9 at 25°C 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, Sigma Prod. No. G-5251.)

C. 20 mM β-Nicotinamide Adenine Dinucleotide, Oxidized Form Solution (β-NAD)
(Prepare 1 ml in deionized water using β-Nicotinamide Adenine Dinucleotide, Sigma prod. No. N-7004.)

D. 10 mM Adenosine 5'-Diphosphate Solution (ADP)[1]
(Prepare 1 ml in deionized water using Adenosine 5'-Diphosphate, Sodium Salt, Sigma Prod. No. A-2754. **Prepare Fresh.**)

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REAGENTS: (continued)

E. 50 mM Magnesium Sulfate Solution ($MgSO_4$)
(Prepare 10 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)

F. 400 mM Glycine Solution (Glycine)
(Prepare 25 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126.)

G. Glyceraldehyde-3-Phosphate Dehydrogenase Solution (GAPDH)
(Immediately before use, prepare a solution containing 20 units/ml of Glyceraldehyde 3-Phosphate Dehydrogenase, Sigma Prod. No. G-5537, 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 (β-NAD)	0.05	0.05
Reagent D (ADP)	0.05	0.05
Reagent E ($MgSO_4$)	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 A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent H (3-PGK)	0.10	------
Reagent A (Buffer)	------	0.10

Immediately mix by inversion and record the increase in A_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/min using the maximum linear rate for both the Test and Blank.

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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 assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm
0.1 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of 1,3-diphosphoglycerate to 3-phosphoglycerate per minute at pH 6.9 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 50 mM potassium phosphate, 0.83 mM glyceraldehyde-3-phosphate, 0.3 mM 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.

REFERENCE:

Bücher, T. (1955) *Methods in Enzymology*, Volume I, 415-422

NOTES:

1. Adenosine 5'-Diphosphate degrades to adenosine 5'-monophosphate which is an inhibitor of 3-phosphoglyceric phosphokinase. Therefore adenosine 5'-diphosphate solutions should be prepared directly before use.

2. This assay is based on the cited reference.

NOTES: (continued)

3. Glyceraldehyde-3-Phosphate Dehydrogenase Unit Definition: One unit will reduce 1.0 µmole of 3-phosphoglycerate to D-glyceraldehyde-3-phosphate per minute in a coupled system with 3-phosphoglyceric phosphokinase at pH 7.6 at 25°C.

4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.