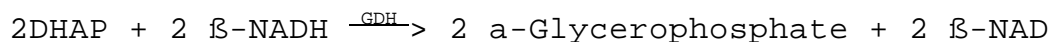
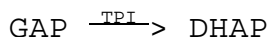
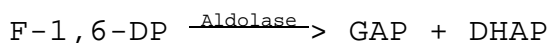
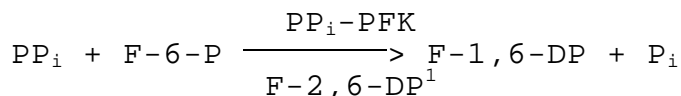


**Enzymatic Assay of FRUCTOSE-6-PHOSPHATE KINASE,
PYROPHOSPHATE DEPENDENT
(EC 2.7.1.90)
from Potato Tubers**

PRINCIPLE:



Abbreviations used:

PP_i = Pyrophosphate

F-6-P = D-Fructose 6-Phosphate

F-2,6-DP = Fructose 2,6-Diphosphate

PP_i-PFK = Fructose-6-Phosphate Kinase,
Pyrophosphate Dependent

F-1,6-DP = D-Fructose 1,6-Diphosphate

P_i = Inorganic Phosphate

GAP = D-Glyceraldehyde 3-Phosphate

TPI = Triosephosphate Isomerase

DHAP = Dihydroxyacetone Phosphate

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

GDH = α-Glycerophosphate Dehydrogenase

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

CONDITIONS: T = 30°C, pH 8.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 75 mM Tris HCl Buffer with 7.5 mM Magnesium Chloride, pH 8.0 at 30°C
(Prepare 100 ml in deionized water using Trizma, Sigma Prod. No. T-1503 and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250. Adjust to pH 8.0 at 30°C with 1 M HCl.)

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REAGENT: (continued)

- B. 75 mM D-Fructose 6-Phosphate Solution (F-6-P)
(Prepare 10 ml in deionized water using D-Fructose 6-Phosphate, Dipotassium Salt, Sigma Prod. No. F-1502.)
- C. 4.3 mM β -Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β -NADH)
(Dissolve the contents of one 10 mg vial of β -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-110, in the appropriate volume of Reagent A. **PREPARE FRESH.**)
- D. Aldolase Enzyme Solution
(Immediately before use, prepare a solution containing 10 units/ml of Aldolase, Sigma Prod. No. A-7145 in cold deionized water.)
- E. α -Glycerophosphate Dehydrogenase/Triosephosphate Isomerase Enzyme Solution² (α -GDH/TPI)
(Immediately before use, prepare a solution containing 10 α -GDH units/ml of α -Glycerophosphate Dehydrogenase-Triosephosphate Isomerase Type X from Rabbit Muscle, Sigma Prod. No. G-6755 in cold deionized water.)
- F. 75 mM Pyrophosphate Buffer, pH 8.0 at 30°C (PP_i)
(Prepare 10 ml in deionized water using Sodium Pyrophosphate, Decahydrate, Sigma Prod. No. S-9515. Adjust to pH 8.0 at 30°C with 1 M HCl.)
- G. 30 μ M Fructose 2,6-Diphosphate Solution (F-2,6-DP)
(Prepare 1 ml in Reagent A using D-Fructose 2,6-Diphosphate, Sodium Salt, Sigma Prod. No. F-7006.)
- H. 175 mM Glucose 6-Phosphate Solution (G-6-P)
(Prepare 5 ml in deionized water using D-Glucose 6-Phosphate, Monosodium Salt, Sigma Prod. No. G-7879.)
- I. Fructose-6-Phosphate Kinase, Pyrophosphate Dependent Enzyme Solution (PP_i-PFK)
(Immediately before use, prepare a solution containing 0.2 - 0.5 unit/ml of Fructose-6-Phosphate Kinase, Pyrophosphate Dependent, in cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.00	2.00
Reagent B (F-6-P)	0.10	0.10
Reagent C (β-NADH)	0.10	0.10
Reagent D (Aldolase)	0.10	0.10
Reagent E (α-GDH/TPI)	0.10	0.10
Reagent G (F-2,6-DP)	0.10	0.10
Reagent H (G-6-P)	0.30	0.30
Reagent I (PP _i -PFK))	0.03	-----
Deionized Water	0.07	0.10

Mix by inversion, and equilibrate to 30°C for 5 minutes. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent F (PP _i)	0.10	0.10
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Immediately mix by inversion and record the decrease in A_{340nm} for approximately 5 minutes. Obtain the r A_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340nm}/\text{min Test} - r A_{340nm}/\text{min Blank})(3)(df)}{(6.22)(2)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

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

2 = Factor accounting for 2 moles of β-NADH oxidized per mole of pyrophosphate converted

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 convert 1.0 μ mole of pyrophosphate and fructose 6-phosphate to fructose 1,6-diphosphate and inorganic phosphate per minute at pH 8.0 at 30°C in the presence of 1 μ M fructose 2,6-diphosphate and 17 mM glucose 6-phosphate in a coupled assay system using aldolase, α -glycerophosphate dehydrogenase and triosephosphate isomerase.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 55 mM Tris, 5.5 mM magnesium chloride, 17 mM D-glucose-6-phosphate, 2.5 mM D-fructose-6-phosphate, 0.14 mM β -nicotinamide adenine dinucleotide, reduced form, 2.5 mM pyrophosphate, 1 unit aldolase, 1 unit α -glycerophosphate dehydrogenase, 8 units of triosephosphate isomerase, 0.006 - 0.015 unit fructose-6-phosphate kinase, pyrophosphate dependent, and 1 μ M fructose 2,6-diphosphate.

REFERENCE:

Van Schaftingen, E., Lederer, B., Bartrons, R., and Hers, H.-G. (1982) *European Journal of Biochemistry*, **129**, 191-195

NOTES:

1. Fructose 2,6-Diphosphate is an activator of Fructose-6-Phosphate Kinase, Pyrophosphate Dependent from Potato Tubers.
2. The Triosephosphate Isomerase activity is approximately 8 fold that of the α -Glycerophosphate Dehydrogenase activity.
3. Aldolase Unit Definition: One unit will convert 1.0 μ mole of fructose 1,6-diphosphate to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate per minute at pH 7.4 at 25°C.

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NOTES: (continued)

4. a-Glycerophosphate Dehydrogenase Unit Definition: One unit will convert 1.0 μ mole of dihydroxyacetone phosphate to a-glycerophosphate per minute at pH 7.4 at 25°C.
5. Triosephosphate Isomerase Unit Definition: One unit will convert 1.0 μ mole of D-glyceraldehyde 3-phosphate to dihydroxyacetone phosphate per minute at pH 7.6 at 25°C.
6. This assay is based on the cited reference.
7. 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.