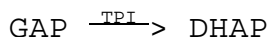
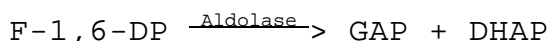
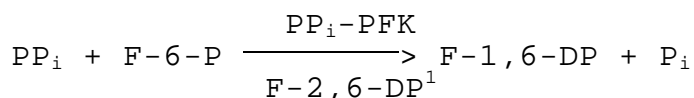


**Enzymatic Assay of FRUCTOSE-6-PHOSPHATE KINASE,
PYROPHOSPHATE DEPENDENT
(EC 2.7.1.90)
from Mung Bean**

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

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Imidazole HCl Buffer with 1 mM Magnesium Chloride and 0.2 mM Ethylenediaminetetraacetic Acid, pH 7.6 at 30°C
(Prepare 100 ml in deionized water using Imidazole, Sigma Prod. No. I-0250, Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250, and Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS. Adjust to pH 7.6 at 30°C with 1 M HCl.)

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REAGENT: (continued)

- B. 100 mM D-Fructose-6-Phosphate Solution (F-6-P)
(Prepare 10 ml in Reagent A using D-Fructose 6-Phosphate, Dipotassium Salt, Sigma Prod. No. F-1502.)
- C. 5.0 mM β -Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β -NADH)
(Dissolve the contents of one 5 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 50 units/ml of Aldolase, Sigma Prod. No. A-7145 in cold Reagent A.)
- E. α -Glycerophosphate Dehydrogenase/Triosephosphate Isomerase Enzyme Solution¹ (α -GDH/TPI)
(Immediately before use, prepare a solution containing 50 α -GDH units/ml of α -Glycerophosphate Dehydrogenase-Triosephosphate Isomerase Type X from Rabbit Muscle, Sigma Prod. No. G-6755 in cold Reagent A.)
- F. 30 mM Pyrophosphate Solution (PP_i)
(Prepare 10 ml in Reagent A using Pyrophosphate, Disodium, Sigma Prod. No. P-8135.)
- G. 30 μ M Fructose 2,6-Diphosphate Solution² (F-2,6-DP)
(Prepare 1 ml in Reagent A using D-Fructose 2,6-Diphosphate, Sodium Salt, Sigma Prod. No. F-7006.)
- H. Fructose-6-Phosphate Kinase, Pyrophosphate Dependent Enzyme Solution (PP_i-PFK)
(Immediately before use, prepare a solution containing 0.02 - 0.07 unit/ml in deionized water.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	1.45	1.45
Reagent B (F-6-P)	1.00	1.00

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PROCEDURE: (continued)

	<u>Test</u>	<u>Blank</u>
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 (PP_i -PFK)	0.10	-----
Deionized Water	-----	0.10

Mix by inversion and equilibrate to 30°C. 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 $r A_{340nm}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340nm}/\text{min Test} - r A_{340nm}/\text{min Blank})(3.05)(df)}{(6.22)(2)(0.1)}$$

3.05 = 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 7.6 at 30°C in a coupled system with aldolase, α -glycerophosphate dehydrogenase, triosephosphate isomerase, and 1 μ mole fructose 2,6-diphosphate.

FINAL ASSAY CONCENTRATION:

In a 3.05 ml reaction mix, the final concentrations are 97 mM imidazole buffer, 1 mM magnesium chloride, 0.2 mM ethylenediaminetetraacetic acid, 33 mM D-fructose-6-phosphate, 0.16 mM β -nicotinamide adenine dinucleotide, reduced form, 0.98 mM pyrophosphate, 5 units aldolase, 5 units α -glycerophosphate dehydrogenase, 40 units of triosephosphate isomerase, 0.002 - 0.007 unit fructose-6-phosphate kinase, pyrophosphate dependent, and 0.98 μ M fructose 2,6-diphosphate.

REFERENCE:

Sabularse, D.C. and Anderson, R.L. (1981) *Biochemical and Biophysical Research Communications* **103**, 848-855

NOTES:

1. Fructose 2,6-Diphosphate is an activator of Fructose-6-Phosphate Kinase, Pyrophosphate Dependent from Mung Bean.
2. The Triosephosphate Isomerase activity is approximately 8 fold that of the α -Glycerophosphate Dehydrogenase.
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.
4. α -Glycerophosphate Dehydrogenase Unit Definition: One unit will convert 1.0 μ mole of dihydroxyacetone phosphate to α -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.

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NOTES: (continued)

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.