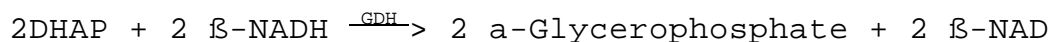
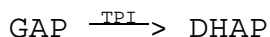
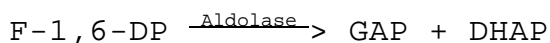
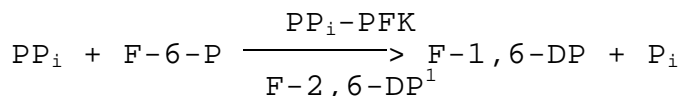


**Enzymatic Assay of FRUCTOSE-6-PHOSPHATE KINASE,  
PYROPHOSPHATE DEPENDENT  
(EC 2.7.1.90)  
from Potato Tubers**

**PRINCIPLE:**



Abbreviations used:

PP<sub>i</sub> = Pyrophosphate

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

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

PP<sub>i</sub>-PFK = Fructose-6-Phosphate Kinase,  
Pyrophosphate Dependent

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

P<sub>i</sub> = 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<sub>340nm</sub>, 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  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form Solution ( $\beta$ -NADH)  
(Dissolve the contents of one 10 mg vial of  $\beta$ -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.  $\alpha$ -Glycerophosphate Dehydrogenase/Triosephosphate Isomerase Enzyme Solution<sup>2</sup> ( $\alpha$ -GDH/TPI)  
(Immediately before use, prepare a solution containing 10  $\alpha$ -GDH units/ml of  $\alpha$ -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<sub>i</sub>)  
(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  $\mu$ 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<sub>i</sub>-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 <sub>i</sub> -PFK))	0.03	-----
Deionized Water	0.07	0.10

Mix by inversion, and equilibrate to 30°C for 5 minutes. Monitor the A<sub>340nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent F (PP <sub>i</sub> )	0.10	0.10
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Immediately mix by inversion and record the decrease in A<sub>340nm</sub> for approximately 5 minutes. Obtain the r A<sub>340nm</sub>/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

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

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  $\mu$ 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  $\mu$ M fructose 2,6-diphosphate and 17 mM glucose 6-phosphate in a coupled assay system using aldolase,  $\alpha$ -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  $\beta$ -nicotinamide adenine dinucleotide, reduced form, 2.5 mM pyrophosphate, 1 unit aldolase, 1 unit  $\alpha$ -glycerophosphate dehydrogenase, 8 units of triosephosphate isomerase, 0.006 - 0.015 unit fructose-6-phosphate kinase, pyrophosphate dependent, and 1  $\mu$ 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  $\alpha$ -Glycerophosphate Dehydrogenase activity.
3. Aldolase Unit Definition: One unit will convert 1.0  $\mu$ 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  $\mu$ 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  $\mu$ 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.**