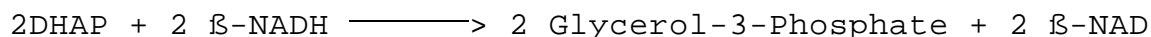
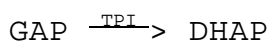
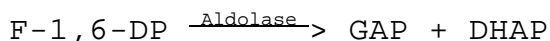


**Enzymatic Assay of FRUCTOSE-6-PHOSPHATE KINASE,
PYROPHOSPHATE DEPENDENT
(EC 2.7.1.90)**

PRINCIPLE:



Abbreviations used:

PPi = Pyrophosphate

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

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

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

Pi = Inorganic Phosphate

GAP = D-Glyceraldehyde-3-Phosphate

TPI = Triosephosphate Isomerase

DHAP = Dihydroxyacetone Phosphate

GDH = α-Glycerophosphate Dehydrogenase

β-NADH = β-Nicotinamide Adenine Dinucleotide (reduced form)

β-NAD = β-Nicotinamide Adenine Dinucleotide (oxidized form)

CONDITIONS: T = 30°C, pH 7.4, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 150 mM Imidazole Buffer with 1 mM Magnesium Chloride, pH 7.4 at 30°C
(Prepare 100 ml in deionized water using Imidazole, Prod. No. I-0250 and Magnesium Chloride, Hexahydrate, Prod. No. M-0250. Adjust to pH 7.4 at 30°C with 1 M HCl.)
- B. 90 mM D-Fructose-6-Phosphate Solution (F-6-P)
(Prepare 10 ml in Reagent A using D-Fructose 6-Phosphate, Dipotassium Salt, Prod. No. F-1502.)

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REAGENT: (continued)

- C. 6.4 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, Stock No. 340-105, in 1 ml of Reagent A. **PREPARE FRESH.**)
- D. Aldolase Enzyme Solution
(Immediately before use, prepare a solution containing 50 units/ml of Aldolase, Prod. No. A-7145 in cold Reagent A.)
- E. α -Glycerophosphate Dehydrogenase/Triosephosphate Isomerase Enzyme Solution (α -GDH/TPI)¹
(Immediately before use, prepare a solution containing 25 α -GDH units/ml of α -Glycerophosphate Dehydrogenase Triosephosphate Isomerase Type X from Rabbit Muscle, Prod. No. G-6755 in cold Reagent A.)
- F. 100 mM Pyrophosphate Solution, pH 7.4 at 30°C (PPi)
(Prepare 10 ml in Reagent A using Pyrophosphate, Disodium, Prod. No. P-8135. Adjust to pH 7.4 at 30°C with either 1 M HCl or 1 M NaOH.)
- G. Fructose-6-Phosphate Kinase, Pyrophosphate Dependent Enzyme Solution (PPi-PFK)
(Immediately before use, prepare a solution containing 0.1 - 0.5 units/ml in deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.50	2.50
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 F (PPi)	0.05	0.05

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PROCEDURE: (continued)

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

Reagent G (PPi-PFK)	0.050	-----
Deionized Water	-----	0.050

Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{340\text{nm}}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3)(\text{df})}{(6.22)(2)(0.05)}$$

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

0.05 = Volume (in milliliters) 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 μ mole of pyrophosphate and fructose 6-phosphate to fructose 1,6-diphosphate and inorganic phosphate per minute at pH 7.4 at 30°C in a coupled system with aldolase, glycerophosphate dehydrogenase and triosephosphate isomerase.

FINAL ASSAY CONCENTRATION:

In a 3.0 ml reaction mix, the final concentrations are 147.5 mM imidazole buffer, 0.98 mM magnesium chloride, 3.0 mM D-fructose-6-phosphate, 0.21 mM β -NADH, 1.67 mM pyrophosphate, 5.0 units aldolase, 2.5 units a-GDH/TPI, and .005 - 0.025 units PPi-PFK.

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NOTES:

1. Contains not less than 125 a-GDH units/mg protein and 1250 TPI units/mg protein.
2. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.