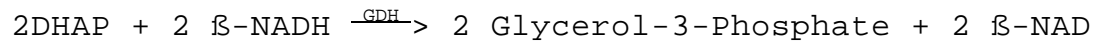
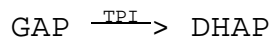
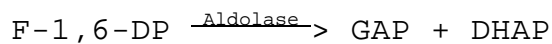
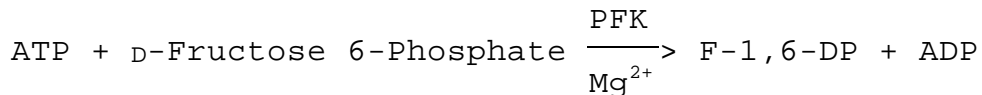


**Enzymatic Assay of FRUCTOSE-6-PHOSPHATE KINASE
(EC 2.7.1.11)
from Rabbit Liver**

PRINCIPLE:



Abbreviations used:

ATP = Adenosine 5'-Triphosphate

PFK = Fructose-6-Phosphate Kinase

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

ADP = Adenosine 5'-Diphosphate

GAP = D-Glyceraldehyde 3-Phosphate

DHAP = Dihydroxyacetone Phosphate

TPI = Triosephosphate Isomerase

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. 60 mM Tris HCl Buffer, pH 8.0 at 30°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 30°C with 1 M HCl.)
- B. 30 mM DL-Dithiothreitol Solution (DTT)
(Prepare 10 ml in deionized water using DL-Dithiothreitol, Sigma Prod. No. D-0632.)
- C. 60 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 10 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-6144.)

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REAGENTS: (continued)

- D. 6 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. 340-105, in the appropriate volume of deionized water.)
- E. 750 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
- F. Aldolase Enzyme Solution (Aldolase)
(Immediately before use, prepare a solution containing 100 units/ml of Aldolase, Sigma Prod. No. A-1893, in cold deionized water.)
- G. α -Glycerophosphate Dehydrogenase/Triosephosphate Isomerase Enzyme Solution¹ (GDH/TPI)
(Immediately before use, prepare a solution containing 100 GDH units/ml using α -Glycerophosphate Dehydrogenase-Triosephosphate Isomerase, Sigma Prod. No. G-6755, in cold deionized water.)
- H. 120 mM D-Fructose 6-Phosphate Solution (F-6P)
(Prepare 10 ml in deionized water using D-Fructose 6-Phosphate, Disodium Salt, Sigma Prod. No. F-3627.)
- I. Fructose-6-Phosphate Kinase Enzyme Solution (PKF)
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml in cold 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 (DTT)	0.10	0.10
Reagent C (ATP)	0.10	0.10
Reagent D (β -NADH)	0.10	0.10
Reagent E ($MgCl_2$)	0.02	0.02
Reagent F (Aldolase)	0.02	0.02
Reagent G (GDH/TPI)	0.01	0.01
Reagent I (PFK)	0.05	-----
Deionized Water	-----	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:

	<u>Test</u>	<u>Blank</u>
Reagent H (F-6P)	0.10	0.10

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

CALCULATIONS:

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.05 = Volume (in milliliter) of enzyme used

2 = Conversion factor accounting for 2 moles of β -NADH oxidized per mole of D-fructose-1,6-diphosphate produced

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

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

UNIT DEFINITION:

One unit will convert 1.0 μmole of fructose 6-phosphate and ATP to fructose 1,6-diphosphate and ADP per minute at pH 8.0 at 30°C in a coupled system with aldolase, glycerophosphate dehydrogenase and triosephosphate isomerase.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 50 mM Tris, 1 mM DL-dithiothreitol, 2 mM adenosine 5'-triphosphate, 0.2 mM β -nicotinamide adenine dinucleotide, reduced form, 5 mM magnesium chloride, 2 units aldolase, 1 unit α -glycerophosphate dehydrogenase, 8 units triosephosphate isomerase, 4 mM fructose 6-phosphate, and 0.025 - 0.05 unit fructose-6-phosphate kinase.

REFERENCE:

Massey, T.H. and Deal, Jr., W.C. (1973) *Journal of Biological Chemistry* **248**, 56-62

NOTES:

1. The Triosephosphate Isomerase activity is approximately 8 fold that of the α -Glycerophosphate Dehydrogenase activity.
2. 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.
3. 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.
4. 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.
5. This assay is based on the cited reference.
6. 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.