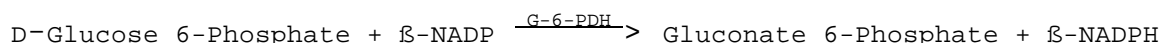
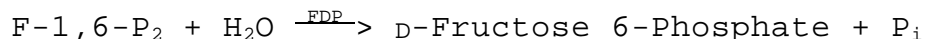


**Enzymatic Assay of D-FRUCTOSE-1,6-DIPHOSPHATASE  
(EC 3.1.3.11)**

**PRINCIPLE:**



Abbreviations used:

F-1,6-P<sub>2</sub> = D-Fructose 1,6-Diphosphate

P<sub>i</sub> = Inorganic Phosphate

FDP = D-Fructose-1,6-Diphosphatase

PGI = Phosphoglucose Isomerase

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

G-6-PDH = Glucose-6-Phosphate Dehydrogenase

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

**CONDITIONS:** T = 25°C, pH 9.5, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 100 mM Glycine Buffer, pH 9.5 at 25°C  
(Prepare 100 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126. Adjust to pH 9.5 at 25°C with 1 M NaOH.)
- B. 41 mM D-Fructose 1,6-Diphosphate Solution (F-1,6-P<sub>2</sub>)  
(Prepare 1 ml in deionized water using D-Fructose 1,6-Diphosphate, Tetra(cyclohexylammonium) Salt, Sigma Prod. No. F-0752.)
- C. 51 mM Manganese Chloride Solution (MnCl<sub>2</sub>)  
(Prepare 1 ml in deionized water using Manganese Chloride, Tetrahydrate, Sigma Prod. No. M-3634.)

**Enzymatic Assay of D-FRUCTOSE-1,6-DIPHOSPHATASE  
(EC 3.1.3.11)**

**REAGENTS:**

- D. Phosphoglucose Isomerase Enzyme Solution (PGI)  
(Immediately before use, prepare a solution containing 700 units/ml of Phosphoglucose Isomerase, Sigma Prod. No. P-9010, in cold deionized water.)
- E. Glucose-6-Phosphate Dehydrogenase Enzyme Solution (G-6-PDH)  
(Immediately before use, prepare a solution containing 300 units/ml of Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. G-8878, in cold deionized water.)
- F. 12 mM  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form, Solution ( $\beta$ -NADP)  
(Prepare 2 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505.)
- G. D-Fructose-1,6-Diphosphatase Enzyme Solution (FDP)  
(Immediately before use, prepare a solution containing 1.5 - 3.0 units/ml of D-Fructose-1,6-Diphosphatase in cold Reagent A.)

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.74	2.74
Reagent B (F-1,6-P <sub>2</sub> )	0.10	0.10
Reagent C (MnCl <sub>2</sub> )	0.02	0.02
Reagent D (PGI)	0.02	0.02
Reagent E (G-6-PDH)	0.01	0.01

Mix by inversion and equilibrate to 25°C. Monitor the A<sub>340nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent F ( $\beta$ -NADP)	0.10	0.10
Reagent G (FDP)	0.02	-----
Reagent A (Buffer)	-----	0.02

Immediately mix by inversion and record the increase in A<sub>340nm</sub> for approximately 5 minutes. Obtain the  $\Delta A_{340nm}/\text{minute}$  using the maximum linear rate for both the Test and Blank.

**Enzymatic Assay of D-FRUCTOSE-1,6-DIPHOSPHATASE  
(EC 3.1.3.11)**

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(3.01)(\text{df})}{(6.22)(0.02)}$$

3.01 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of  $\beta$ -NADPH at  
340 nm

0.02 = Volume (in milliliter) of enzyme used

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

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

**UNIT DEFINITION:**

One unit will convert 1.0  $\mu$ mole of fructose 1,6-diphosphate to fructose 6-phosphate and inorganic phosphate per minute at pH 9.5 at 25°C.

**FINAL ASSAY CONCENTRATION:**

In a 3.01 ml reaction mix, the final concentrations are 92 mM glycine, 1.4 mM fructose 1,6-diphosphate, 0.34 mM manganese chloride, 0.4 mM  $\beta$ -nicotinamide adenine dinucleotide phosphate, 14 units phosphoglucose isomerase, 3 units glucose-6-phosphate dehydrogenase and 0.03 - 0.06 unit fructose-1,6-diphosphatase.

**REFERENCE:**

Pontremoli, S., Traniello, S., Luppis, B., and Wood, W.A., (1965) *Journal of Biological Chemistry* **240**, 3459-3463

**NOTES:**

1. Phosphoglucose Isomerase Unit Definition: One unit will convert 1.0  $\mu$ mole of fructose 6-phosphate to glucose 6-phosphate per minute at pH 7.4 at 25°C.
2. Glucose-6-Phosphate Dehydrogenase Unit Definition: One unit will oxidize 1.0  $\mu$ mole of D-glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of  $\beta$ -NADP at pH 7.4 at 25°C.

**Enzymatic Assay of D-FRUCTOSE-1,6-DIPHOSPHATASE  
(EC 3.1.3.11)**

**NOTES:**

3. This assay is based on the cited reference.
4. 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.**