Enzymatic Assay of $_D$-FRUCTOSE DEHYDROGENASE
(EC 1.1.99.11)

PRINCIPLE:

$_D$-Fruc + 2 $K_3Fe(CN)_6$ $\xrightarrow{\text{D-Fructose Dehydrogenase}}$ 5-K-$_D$-Fruc + 2 $K_4Fe(CN)_6$

2 $K_4Fe(CN)_6$ + Ferric Sulfate ——————> 2 Prussian Blue + 3 K_2SO_4

Abbreviations used:
$_D$-Fruc = $_D$(-)Fructose
5-K-$_D$-Fruc = 5-Keto-$_D$-Fructose

CONDITIONS: T = 37°C, pH = 4.5, A_{660nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 200 mM Sodium Phosphate with 0.23% (v/v) Triton[1] X-100 Solution
(Prepare 100 ml in deionized water using Sodium Phosphate, Dibasic, Sigma Prod. No. S-0876, and Triton[1] X-100, Sigma Stock No. X-100.)

B. 100 mM Citric Acid Solution
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Anhydrous, Sigma Prod. No. C-0759.)

C. McIlvaine Buffer with 0.1% Triton[1] X-100, pH 4.5 at 37°C
(Prepare by combining 90 ml of Reagent A with 110 ml of Reagent B. Adjust the pH to 4.5 at 37°C with either Reagent A or Reagent B as needed.)

D. 1 M $_D$-Fructose Solution ($_D$-Fructose)
(Prepare 10 ml in Reagent C using $_D$(-)Fructose, Sigma Prod. No. F-0127.)

Enzymatic Assay of D-FRUCTOSE DEHYDROGENASE (EC 1.1.99.11)

REAGENTS: (continued)

E. 100 mM Potassium Ferricyanide Solution ($K_3Fe(CN)_6$) (Prepare 10 ml in Reagent C using Potassium Ferricyanide, Sigma Prod. No. P-8131.)

F. 0.5% (w/v) Ferric Sulfate with 0.3% (w/v) Sodium Dodecyl Sulfate and 8.1% (v/v) Phosphoric Acid Solution (Stop Soln)
(Prepare 50 ml in deionized water using Ferric Sulfate, Sigma Prod. No. F-1135, Lauryl Sulfate, Sodium Salt,
Sigma Prod. No. L-4509, and Phosphoric Acid, P-6560.)

G. D-Fructose Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 1.0 - 2.0 units/ml of D-Fructose Dehydrogenase in cold Reagent C.)

PROCEDURE:

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

	Test	Blank
Reagent C (Buffer)	0.70	0.80
Reagent D (D-Fructose)	0.10	------
Reagent G (Enzyme Solution)	0.10	0.10

Mix by swirling and equilibrate to 37°C. Then add:

Reagent E ($K_3Fe(CN)_6$)	0.10	0.10

Immediately mix by swirling and incubate for exactly 5 minutes at 37°C. Then add:

Reagent F (Stop Soln.)	0.50	0.50

Immediately mix by swirling and incubate for 20 minutes. Then add:

Deionized Water	3.50	3.50

Mix by swirling and transfer the solutions to suitable cuvettes. Record the A_{660nm} for both the Test and Blank using a suitable spectrophotometer.

Enzymatic Assay of $_D$-FRUCTOSE DEHYDROGENASE
(EC 1.1.99.11)

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{660nm}\ \text{Test} - A_{660nm}\ \text{Blank})\ (5)\ (df)}{(2)\ (2)\ (5)\ (0.1)}$$

5 = Total volume (in milliliters) of assay
df = Dilution factor
2 = Millimolar extinction coefficient of Prussian Blue at 660 nm
2 = Two moles of Prussian Blue produced per mole of $_D$-Fructose oxidized
5 = Time (in minutes) of assay as per the Unit Definition
0.1 = 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 µmole of $_D$-fructose to 5-keto-$_D$-fructose per minute at pH 4.5 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 90 mM sodium phosphate, 55 mM citric acid, 0.1% (v/v) Triton[1] X-100, 100 mM $_D$(-)fructose, 10 mM potassium ferricyanide and 0.1 - 0.2 unit $_D$-fructose dehydrogenase.

REFERENCES:

Ameyama, M. (1982) *Methods in Enzymology* **89**, Part D, 20-29.

NOTES:

1. Triton is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc.

2. This assay is based on the cited reference.

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

Enzymatic Assay of D-FRUCTOSE DEHYDROGENASE (EC 1.1.99.11)

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