Enzymatic Assay of DEXTRAN SUCRASE (EC 2.4.1.5)

PRINCIPLE:

$$\text{Sucrose} + (1,6\text{-a-D-glucosyl})_n \xrightarrow{\text{Dextran Sucrase}} \text{Fructose} + (1,6\text{-a-D-glucosyl})_{n+1}$$

CONDITIONS: T = 30°C, pH = 5.2, A_{546nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 50 mM Sodium Acetate Buffer with 1 mM Calcium Chloride,
pH 5.2 at 30°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625 and Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881. Adjust to pH 5.2 at 30°C using 1 M HCl.)

B. 12.5% (w/v) Sucrose Substrate Solution (Sucrose)
(Prepare 10 ml in Reagent A using Sucrose, Sigma Prod. No. S-9378.)

C. Dextran Sucrase Enzyme Solution (Enz Soln)
(Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Dextran Sucrase in cold Reagent A.)

D. 26 mM Copper Sulfate, 1.3 M Sodium Sulfate, 225 mM Sodium Carbonate, 190 mM Sodium Bicarbonate and 43 mM Sodium Potassium Tartrate Solution (Copper Soln)
(Prepare 1 liter in deionized water using Cupric Sulfate Pentahydrate, Sigma Prod. No. C-7631, Sodium Bicarbonate, Sigma Prod. No. S-8875, Sodium Sulfate, Anhydrous, Sigma Prod. No. S-9627, Sodium Carbonate, Anhydrous, Sigma Prod. No. S-2127, and Sodium Potassium Tartrate Tetrahydrate, Sigma Prod. No. S-2377.)[1]

E. 40 mM Molybdic Acid, 19 mM Arsenic acid and 756 mM Sulfuric Acid Solution (Ars-Mol)
(Prepare 1 liter in deionized water using Molybdic Acid, Ammonium Salt Tetrahydrate, Sigma Prod. No. M-0878, Arsenic Acid, Sodium Salt, Sigma Prod. No. A-

6756 and Sulfuric Acid, Sigma Prod. No. S-1526.)[2]

Enzymatic Assay of DEXTRAN SUCRASE (EC 2.4.1.5)

REAGENTS: (continued)

F. 1.39 mM Fructose Standard Solution (Std Soln) (Prepare 10 ml in deionized water using Fructose, Sigma Prod. No. F-0127.)

PROCEDURE:

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

	Test	Test Blank	Std 1	Std 2	Std 3	Std 4	Std 5	Std Blank
Reagent B (Sucrose)	0.40	0.40	----	----	----	----	----	----
Reagent F (Std Soln)	----	----	0.10	0.20	0.30	0.40	0.50	----
Deionized Water	----		----		0.40		0.30	0.20
								0.10

								0.50

Mix by swirling and equilibrate to 30°C. Then add:

Reagent C (Enz Soln)	0.10	----	----	----	----	----	----	----

Immediately mix by swirling and incubate at 30°C for exactly 15 minutes. Then add:

Reagent D (Copper Soln)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Reagent C (Enz Soln)	----	0.10	----	----	----	----	----	----

Mix by swirling. Place a marble over the top of each tube and transfer the tubes to a boiling water bath. Incubate for 15 minutes. Remove from the boiling water bath and allow to cool to room temperature. Then add:

Reagent E (Ars-Mol)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

Shake or vortex until the foaming stops and any precipitate present is dissolved. Then add:

Deionized Water	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00

Mix by swirling and transfer the solutions to cuvettes. Record the A_{546nm} for the Test, Blank and Standards using a

suitable spectrophotometer.

Enzymatic Assay of DEXTRAN SUCRASE
(EC 2.4.1.5)

CALCULATIONS:

Standard Curve:

ΔA_{546nm} Std = A_{546nm} Std - A_{546nm} Std Blank

Prepare a standard curve by plotting the ΔA_{546nm} Standard versus the µmoles of fructose.

Sample Determination

ΔA_{546nm} Sample = A_{546nm} Test - A_{546nm} Test Blank

Determine the µmoles of fructose using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of fructose liberated})(df)}{(15)(0.1)}$$

df = Dilution factor
15 = Time (in minutes) of the assay as per the Unit Definition
0.1 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will liberate 1.0 µmole of fructose from sucrose per minute at pH 5.2 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 0.50 ml reaction mix, the final concentrations are 50 mM sodium acetate, 1 mM calcium chloride, 10% (w/v) sucrose and 0.01 - 0.02 unit dextran sucrase.

REFERENCE:

Kobayashi, M. and Matsuda, K. (1980) *Biochimica et Biophysica Acta* **614**, 46-62

Enzymatic Assay of DEXTRAN SUCRASE (EC 2.4.1.5)

NOTES:

1. Sodium Sulfate, Sodium Carbonate, and Sodium Potassium Tartrate are dissolved in approximately 500 ml of deionized water. Cupric Sulfate is dissolved in approximately 10 ml of deionized water and is slowly added to the above solution to avoid precipitation. Sodium Bicarbonate is dissolved first in deionized water and then added to the above solution. Dilute the solution to 1 liter. If a precipitate forms, it should be removed by filtration prior to use. Store in an amber bottle and avoid exposure to direct sunlight. Store at room temperature.

2. Molybdic Acid is dissolved in approximately 300 ml of deionized water. Add Sulfuric acid slowly. Caution, this is an exothermic reaction! Arsenic Acid is dissolved in approximately 300 ml of deionized water and is added to the above solution. The solution is diluted to a total volume of 1 liter and incubated at 37°C for 48 - 72 hours. If a precipitate forms, it should be removed by filtration prior to use. Store in an amber bottle and avoid exposure to direct sunlight. The solution expires six months after preparation. Store at room temperature in an exhaust hood.

3. This assay is based on the cited reference.

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