

**Enzymatic Assay of MYCODEXTRANASE
(EC 3.2.1.61)**

PRINCIPLE:

Nigeran ~~Mycodextranase~~ → Nigerose + 4-a-D-Nigerosylglucose

CONDITIONS: T = 37°C, pH = 4.5, A_{540nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 100 mM Sodium Acetate Buffer, pH 4.5 at 37°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 4.5 at 37°C with 1 M HCl.)
- B. 1.25% (w/v) Nigeran Solution (Nigeran)
(Immediately before use, prepare 5 ml in boiling deionized water using Nigeran, Sigma Prod. No. N-2888. Continue boiling until dissolved. Allow to cool to 37°C before using in the assay.)
- C. Mycodextranase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of Mycodextranase in cold deionized water.)
- D. 16 mM Copper Sulfate, 1300 mM Sodium Sulfate, 226 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 Soln)
(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⁾

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REAGENTS: (continued)

F. Glucose Standard Solution (Glucose)
(Use Glucose Standard Solution, Sigma Stock No. 635-100.)

PROCEDURE:

Step 1:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.00	1.00
Deionized Water	0.80	0.90
Reagent C (Enzyme Solution)	0.10	-----

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

Reagent B (Nigeran)	0.10	0.10
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Immediately mix by swirling and incubate at 37° for exactly 10 minutes.

Step 2:

Immediately transfer 1 ml of the reaction mixture into a suitable container containing 1 ml of Reagent D (Copper Soln) as indicated below and proceed with Somogyi's method³ of assaying reducing sugars. Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>	<u>Test</u>	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Std</u>
	<u>Test</u>	<u>Blank</u>						<u>Blank</u>
Test Solution	1.00	---	---	---	---	---	---	---
Test Blank Solution	---	1.00	---	---	---	---	---	---
Deionized Water	---	---	0.97	0.95	0.93	0.90	0.80	1.00
Reagent F (Glucose)	---	---	0.03	0.05	0.07	0.10	0.20	---
Reagent D (Copper Soln)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Immediately mix by inversion. Place a marble over the top of the tubes and transfer the tubes to a boiling water bath. Incubate for 20 minutes. Remove from the boiling water bath and allow to cool to room temperature. Then add:

Reagent E (Ars-Mol Soln) 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00

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PROCEDURE: (continued)

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

	Test	Test Blank	Std 1	Std 2	Std 3	Std 4	Std 5	Std Blank
Deionized Water	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00

Mix and transfer to suitable cuvettes. Obtain the A_{540nm} for Test, Blank and Standards, using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

$$\Delta A_{540nm} \text{ Std} = A_{540nm} \text{ Std} - A_{540nm} \text{ Blank}$$

Prepare a standard curve by plotting the ΔA_{540nm} of the Standard versus the μmoles of glucose liberated.

Sample Determination:

$$\Delta A_{540nm} \text{ Sample} = A_{540nm} \text{ Test} - A_{540nm} \text{ Test Blank}$$

Determine the μmoles of glucose (reducing equivalent) liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of glucose liberated}) (2)}{(10) (0.1)}$$

2 = Volume (in milliliters) of assay

10 = Time of assay (in minutes) as per the Unit Definition

0.1 = Volume (in milliliter) of enzyme used

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

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

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UNIT DEFINITION:

One unit will liberate 1.0 μ mole of reducing sugar (measured as glucose) from nigeran per minute at pH 4.5 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 2.00 ml reaction mix, the final concentrations are 50 mM sodium acetate, 0.063% (w/v) nigeran, and 0.05 - 0.10 unit mycodextranase.

REFERENCES:

Somogyi M., (1952) *J. Biol. Chem.* **195**, 19-23
Somogyi M., (1945) *J. Biol. Chem.* **160**, 61-68
Nelson N., (1944) *J. Biol. Chem.* **153**, 375-380
Reese, E.T. and Mandels, M. (1964) *Canadian Journal of Microbiology* **10**, 103-114

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 100 ml of deionized water and 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! A solution of 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. The method of assaying for the presence of reducing sugars, described here, is that of Somogyi and Nelson

(Somogyi M., 1952, Somogyi M., 1945, and Nelson, N., 1944).

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NOTES: (continued)

4. This assay is based on the cited references.
5. 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.