

**Enzymatic Assay of DEXTRANASE
(EC 3.2.1.11)**

PRINCIPLE:

Dextran Dextranase > Iso-Maltose

CONDITIONS: T = 37°C, pH = 6.0, A_{540nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 100 mM Potassium Phosphate Buffer, pH 6.0 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379. Adjust to pH 6.0 at 37°C with 1 M NaOH.)
- B. 2.0% (w/v) Dextran with 0.01% Thimerosal Solution
(Prepare 25 ml in Reagent A using Dextran, Industrial Grade, average molecular weight 200,000 - 300,000, Prod. No. D-7265, and Thimerosal, Prod. No. T-5125. Adjust to pH 6.0 at 37°C with 100 mM HCl or 100 mM KOH, if necessary.)
- C. Color Reagent Solution containing 30% (w/v) Sodium Potassium Tartrate, and 1.1% (w/v) 3,5-Dinitrosalicylic Acid
(Clr Rgt Soln)
The following components are made separately:
 1. Sodium Potassium Tartrate Solution
(Dissolve 12.0 grams of Sodium Potassium Tartrate, Tetrahydrate, Prod. No. S-2377, in 8.0 ml of 2 M NaOH. Heat in boiling water bath to dissolve, but **DO NOT BOIL.**)
 2. 3,5-Dinitrosalicylic Acid Solution
(Dissolve 438 mg of 3,5-Dinitrosalicylic Acid, Prod. No. D-1510 in 20 ml of deionized water. Heat in boiling water bath to dissolve, but **DO NOT BOIL.**)

**Enzymatic Assay of DEXTRANASE
(EC 3.2.1.11)**

REAGENTS: (continued)

- C. (continued) With stirring, slowly add the Sodium Potassium Tartrate solution to the 3,5-Dinitrosalicylic Acid solution. Dilute to 40 ml with deionized water. If not completely dissolved, the reagents should dissolve when mixed. The solution should be stored in an amber bottle at room temperature. The Color Reagent Solution should be stable for at least 6 months.
- D. 3 mM Maltose Solution (Std Soln)
(Prepare 10 ml in deionized water using Maltose, Monohydrate, Prod. No. M-5885.)
- E. Dextranase Enzyme Solution
(Immediately before use, prepare a solution containing 1 - 2 units/ml of Dextranase in cold deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Dextran)	0.95	0.95

Equilibrate to 37°C. Then add:

Deionized Water	-----	0.05
Reagent E (Enzyme Solution)	0.05	-----

Incubate for exactly 30 minutes at 37°C. Then add:

Reagent C (Clr Rgt Soln)	1.00	1.00
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Mix by inversion and place in a boiling water bath for 15 minutes, then cool and add:

Deionized water	10.00	10.00
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Mix by inversion and record the $A_{540\text{nm}}$ for both the Test and Blank using a suitable spectrophotometer.

**Enzymatic Assay of DEXTRANASE
(EC 3.2.1.11)**

Standard Curve:

A standard curve is made by pipetting (in milliliters) the following reagents into suitable containers:

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Std Blank</u>
Reagent D (Std Soln)	0.10	0.30	0.50	0.70	1.00	0.00
Deionized Water	0.90	0.70	0.50	0.30	----	1.00
Reagent C (Clr Rgt Soln)	1.00	1.00	1.00	1.00	1.00	1.00

Place in a boiling water bath for 15 minutes. Remove the containers, cool in ice bath and then add:

Deionized Water	10.00	10.00	10.00	10.00	10.00	10.00
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Mix by inversion and record the A_{540nm} for the Standards and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

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

Find the slope (M) by plotting the ΔA_{540nm} Standards vs Maltose concentration.

Sample Determination:

Determine the concentration of Maltose liberated using the following equation:

$$\text{mg Maltose released} = \frac{A_{540nm} \text{ Test} - A_{540nm} \text{ Blank}}{(M)}$$

$$\text{Units/mg enzyme} = \frac{(\text{mg Maltose released})}{(30) (\text{mg enzyme/RM})}$$

30 = Time of assay, 30 minutes, while unit definition is per

minute

RM = Reaction Mix, Step 1 (volume = 1.0 ml)

**Enzymatic Assay of DEXTRANASE
(EC 3.2.1.11)**

UNIT DEFINITION:

One unit will liberate 1.0 μ mole of isomaltose (measured as maltose) per minute at pH 6.0 at 37°C, using dextran as substrate.

FINAL ASSAY CONCENTRATIONS:

In a 1 ml reaction mix, the final concentrations are 95 mM potassium phosphate, 2.0% dextran, 0.01% thimerosal and 0.05 - 0.1 units dextranase.

REFERENCES:

Janson, J., and Porath, J. (1966) *Methods in Enzymology* **VIII**, 615.

NOTES:

1. All product 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.