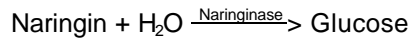


Enzymatic Assay of NARINGINASE

PRINCIPLE:



CONDITIONS: T = 40°C, pH 4.0, $A_{540\text{nm}}$, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 50 mM Sodium Acetate Buffer, pH 4.0 at 40°C
(Prepare 100 ml deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 4.0 at 40°C with 1 M HCL.)
- B. 0.125% Naringin Solution
(Prepare 50 ml in Reagent A using Naringin, Sigma Prod. No. N-1376. Adjust to pH 4.0 at 40°C with 1 M HCl or 1 N NaOH.)
- C. Naringinase Enzyme Solution
(Prepare a solution containing 0.07-0.10 units/ml Naringinase in cold deionized water.)
- D. 16 mM Copper Sulfate, 1.3 M Sodium Sulfate, 226 mM Sodium Carbonate, 190 mM Sodium Bicarbonate and 43 mM Sodium Potassium Tartrate (Copper Solution)
(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 Tartrate Tetrahydrate, Prod. No. S-2377.)²
- E. 40 mM Molybdic Acid, 19 mM Arsenic Acid and 756 mM Sulfuric Acid (Arsenomolybdate Solution)
(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.)³

Enzymatic Assay of NARINGINASE

REAGENTS: (continued)

- F. 250 µg Glucose/ml Standard Solution (Stock)
(Prepare 100 ml each in deionized water using D-(+)-Glucose, Sigma Prod. No. G-8270, with a Class A, 100 ml, volumetric flask.)
- F. Glucose Working Standard Solutions
(Prepare 10 ml each in deionized water containing 25 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml and 250 µg/ml.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Naringin Solution)	4.0	4.0
Deionized Water	-----	1.0

Mix by swirling and equilibrate to 40°C. Then add:

Reagent C (Enzyme Solution)	1.0	-----
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Mix by swirling and incubate at 40°C for 30 minutes.

Immediately transfer 1 ml of reaction mixture into a suitable, container containing 1 ml of Reagent D as indicated below and proceed with Somogyi's method of assay reducing sugars.

	<u>Test</u>	<u>Blank</u>	<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 (Copper Soln)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Test Solution	1.0	----	----	----	----	----	----	----
Blank Solution	----	1.0	----	----	----	----	----	----
Deionized Water	----	----	----	----	----	----	----	1.0
Reagent G (Glucose Soln) ----	----	1.0	1.0	1.0	1.0	1.0	----	----

Mix and incubate in a boiling water bath for 10 minutes. Then add:

Reagent E (Arsenomolybdate Soln)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
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Invert and mix tubes until foaming stops or precipitate is dissolved. Then add:

Deionized Water	10.0	10.0	10.0	10.0	10.0	10.0	10.00	10.00
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Mix by inversion and record the A_{540nm} for Test, Blank and Standards in a suitable spectrophotometer.

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CALCULATIONS:

Standard Curve:

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

Prepare a standard curve by plotting the $A_{540\text{nm}}$ Standard versus glucose concentration. Use the slope to determine the concentration of the Test solution.

$$\text{units/mg Protein} = \frac{(A_{540\text{nm}} \text{ Test} - A_{540\text{nm}} \text{ Blank})(5)}{(30)(\text{mg enzyme/RM}) (1)}$$

30 = Time in minutes of reaction

RM = Reaction Mix

5 = Volume of Step 1 Reaction Mix

1 = Volume transferred from Step 1 to Step 2

UNIT DEFINITION:

One unit will liberate 1.0 μmole of reducing sugar (measured as glucose) from naringin per minute at pH 4.0 at 40°C.

FINAL ASSAY CONCENTRATION:

In a 5.0 ml reaction mix, the final concentrations are 40 mM sodium acetate, 0.1% naringin and 0.07 – 0.10 units naringinase.

REFERENCES:

Somogyi M., (1952) *J. Biol. Chem.*, **195**, 19.

Somogyi M., (1945) *J. Biol. Chem.*, **160**, 61.

Nelson N., (1944) *J. Biol. Chem.*, **153**, 375.

NOTES:

1. The method of assaying for the presence of reducing sugars, described here, is that of Somogyi/Nelson.

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NOTES: (continued)

2. 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.
3. 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. Solution expires six months after preparation. Store at room temperature in an exhaust hood.
4. 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.