

**Enzymatic Assay of CYTOHELICASE
(Laminarinase Activity)**

PRINCIPLE:

Laminarin + H₂O $\xrightarrow{\text{CYTOHELICASE}}$ Reducing Sugar (measured as glucose)

CONDITIONS: T = 50°C, pH = 4.6, A_{540nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 50 mM Citrate Buffer, pH 4.6 at 50°C
(Prepare 50 ml in deionized water using Citric Acid, Free Acid, Anhydrous, Sigma Prod. No. C-0759. Adjust to pH 4.6 at 50°C with 1 M NaOH.)
- B. 0.5% (w/v) Laminarin Substrate Solution (Laminarin)
(Prepare 20 ml in Reagent A using Laminarin, Sigma Prod. No. L-9634.)
- C. Cytohellicase Enzyme Solution
(Immediately before use, prepare a solution containing 0.10 - 0.20 mg solid/ml of Cytohellicase in cold deionized water.)
- D. 15 mM Copper Sulfate, 1.3 M Sodium Sulfate, 226 mM Sodium Carbonate, 190 mM Sodium Bicarbonate and 43 mM 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 CYTOHELICASE
(Laminarinase Activity)**

REAGENTS: (continued)

F. 1 mg/ml Sigma Glucose Standard Solution (Glucose Std)
(Use Glucose Standard Solution, Sigma Stock No. 635-100.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Deionized Water	-----	0.50
Reagent B (Laminarin)	0.50	0.50

Mix by swirling and equilibrate to 50°C. Then add:

Reagent C (Enzyme Solution)	0.50	-----
-----------------------------	------	-------

Mix by swirling and incubate at 50°C for exactly 60 minutes. Then add:

	<u>Test</u>	<u>Test Blank</u>	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std Blank</u>
Deionized Water	---	---	0.90	0.80	0.70	0.60	1.00
Reagent F (Glucose Std)	---	---	0.10	0.20	0.30	0.40	---
Reagent D (Copper Soln)	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Immediately mix by swirling. Place a marble over the top of the tubes and transfer the tubes to a boiling water bath. Incubate for 10 minutes. Remove from the boiling water bath and allow to cool to room temperature. Then add:

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

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

Deionized Water	1.00	1.00	1.00	1.00	1.00	1.00	1.00
-----------------	------	------	------	------	------	------	------

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

**Enzymatic Assay of CYTOHELICASE
(Laminarinase Activity)**

CALCULATIONS:

Standard Curve:

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

Prepare a standard curve by plotting the $\Delta A_{540\text{nm}}$ of the Standard vs the milligrams of glucose.

Sample Determination:

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

Determine the milligrams of glucose liberated using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{(\text{milligrams of glucose liberated}) (\text{df})}{(0.5) (0.5)}$$

df = Dilution factor

0.5 = Milligram of glucose liberated per unit as per the Unit Definition

0.5 = 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 0.5 milligram of glucose per hour at pH 4.6 at 50°C.

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 25 mM citric acid, 0.3% (w/v) laminarin and 0.05 - 0.1 mg solid cytohelicase.

**Enzymatic Assay of CYTOHELICASE
(Laminarinase Activity)**

NOTES:

1. Sodium Sulfate, Sodium Carbonate, and Sodium Potassium Tartrate are dissolved in approximately 50 ml of deionized water. Cupric Sulfate is dissolved in approximately 100 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. Molybdcic 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.

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

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