SIGMA QUALITY CONTROL TEST PROCEDURE

**ProductInformation**

## Enzymatic Assay of LAMINARINASE[1] (EC 3.2.1.6)

**PRINCIPLE:**

Laminarin + $H_2O$ $\xrightarrow{\text{Laminarinase}}$ > Reducing Sugar (measured as glucose)

**CONDITIONS:** T = 37°C, pH = 5.0, $A_{540nm}$, Light path = 1 cm

**METHOD:** Colorimetric

**REAGENTS:**

A. 100 mM Sodium Acetate Buffer, pH 5.0 at 37°C
(Prepare 50 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.0 at 37°C with 1 M HCl.)

B. 2.5% (w/v) Laminarin Substrate Solution (Laminarin)
(Prepare 5 ml in deionized water using Laminarin, Sigma Prod. No. L-9634.)

C. Laminarinase Enzyme Solution
(Immediately before use, prepare a solution containing 0.075 - 0.15 unit/ml of Laminarinase 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 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.)[2]

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.)[3]

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**REAGENTS:** (continued)

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

**PROCEDURE:**

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

| | Test | Blank |
|---|---|---|
| Deionized Water | ------ | 1.00 |
| Reagent A (Buffer) | 3.00 | 3.00 |
| Reagent B (Laminarin) | 1.00 | 1.00 |

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

| | | |
|---|---|---|
| Reagent C (Enzyme Solution) | 1.00 | ------ |

Mix by swirling and incubate at 37°C for exactly 60 minutes.

Pipette[4] (in milliliters) the following reagents into suitable test tubes:

| | Test | Blank | Std 1 | Std 2 | Std 3 | Std 4 | Std Blank |
|---|---|---|---|---|---|---|---|
| Deionized water | 0.90 | 0.90 | 0.90 | 0.80 | 0.70 | 0.60 | 1.00 |
| Test Solution | 0.10 | ---- | ---- | ---- | ---- | ---- | ---- |
| Blank Solution ---- | 0.10 | ---- | ---- | ---- | ---- | ---- | |
| 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 the tubes 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 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 |

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

## Enzymatic Assay of LAMINARINASE[1]
## (EC 3.2.1.6)

**CALCULATIONS:**

Standard Curve:

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

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

Sample Determination:

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

Determine the mg of reducing sugar released as glucose equivalents using the standard curve.

$$\text{Units/ml enzyme} = \frac{(\text{mg of Glucose released})(5)(\text{df})}{(60)(1)(0.1)}$$

5 = Total volume (in milliliters) of assay
df = Dilution factor
60 = Time of assay (in minutes) as per the Unit Definition
1 = Volume (in milliliter) of enzyme used
0.1 = Volume (in milliliter) of Test used in Colorimetric Determination

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

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

**UNIT DEFINITION:**

One unit will liberate 1.0 milligram of reducing sugar (measured as glucose) from laminarin per minute at pH 5.0 at 37°C.

**FINAL ASSAY CONCENTRATIONS:**

In a 5.00 ml reaction mix, the final concentrations are 60 mM sodium acetate, 0.50% (w/v) laminarin and 0.075 - 0.15 unit laminarinase.

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**REFERENCES:**


Somogyi, M. (1952) *Journal of Biological Chemistry* **195**, 19-23

Somogyi, M. (1945) *Journal of Biological Chemistry* **160**, 61-68

Nelson, N. (1944) *Journal of Biological Chemistry* **153**, 375-380


**NOTES:**

1. This assay can also be used as a substrate suitability assay for Laminarin, Sigma Prod. No. L-9634.

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 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.

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. The solution expires six months after preparation. Store at room temperature in an exhaust hood.

4. Reagent D (Copper Solution) and deionized water should already be present in all of the tubes before the Test, Blank, and Reagent F (Glucose Std) are added.

5. This assay is based on the cited references.

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