

**Suitability Assay for LAMINARIN AZURE
as a Substrate for LAMINARINASE**

PRINCIPLE:

Laminarin Azure + H₂O $\xrightarrow{\text{Laminarinase}}$ Soluble Colored Reaction Products

CONDITIONS: T = 37°C, pH = 5.0, A_{595nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 100 mM Sodium Acetate Buffer, pH 5.0 at 37°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.0 at 37°C using 1 M HCl.)
- B. Laminarin Azure (LA)
(Weigh 30 mg of Laminarin Azure, Sigma Prod. No. L-0145, each into 4 suitable vials.)
- C. 95% (v/v) Ethanol (EtOH)
(Prepare 100 ml in deionized water using Ethyl Alcohol, Denatured, Sigma Stock No. 27,074-1.)
- D. Laminarinase Enzyme Solution
(Immediately before use, prepare a solution containing 10 units/ml of Laminarinase, Sigma Prod. No. L-5272, in cold Reagent A.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable vials containing Reagent B (LA):

	<u>Test 1</u>	<u>Test 2</u>	<u>Test 3</u>	<u>Blank</u>
Reagent A (Buffer)	0.90	0.90	0.90	1.00

Incubate for approximately 5 minutes at 37°C. Then add:

Reagent D (Enz Sol)	0.10	0.10	0.10	-----
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PROCEDURE: (continued)

Allow to incubate at 37°C. Then stop the reaction at the following time intervals by adding:

	<u>Test 1</u>	<u>Test 2</u>	<u>Test 3</u>	<u>Blank</u>
Reagent D (EtOH) at				
5 minutes	6.00	-----	-----	-----
10 minutes	-----	6.00	-----	6.00
15 minutes	-----	-----	6.00	-----

Mix by swirling and filter through a 0.45 µm filter. Transfer the filtered solutions to suitable cuvettes and record the A_{595nm} for the Tests and Blank.

CALCULATIONS:

$$\Delta A_{595nm}/\text{min}/\text{unit Laminarinase} = \frac{A_{595nm} \text{ Test} - A_{595nm} \text{ Blank}}{(T)(\text{units of Laminarinase}/\text{RM})}$$

T = Time (in minutes) of assay. Time is 5, 10, or 15 minutes

RM = Reaction Mix

The activity is expressed as the change in absorbance at A_{595nm} per minute per unit Laminarinase. The internal specification for this product is a minimum of 0.05 ΔA_{595nm}/min/unit Laminarinase. Blank values should be less than 0.10 absorbance units at A_{595nm}.

NOTES:

1. 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.