

**Enzymatic Assay of XYLANASE Activity in DRISELASE
(EC 3.2.1.8)**

PRINCIPLE:

Xylan + H₂O $\xrightarrow{\text{Xylanase}}$ Reducing Sugar (measured as glucose)

CONDITIONS: T = 37°C, pH = 4.5, A_{410nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 100 mM Sodium Acetate Buffer, pH 4.5 at 37°C
(Prepare 50 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 4.5 at 37°C with 5 M HCl.)
- B. 2.5% (w/v) Xylan Substrate solution (Xylan)
(Prepare 15 ml in Reagent A using Xylan, Sigma Prod. No. X-0502.)
- C. Driselase (Xylanase) Enzyme Solution
(Immediately before use, prepare a solution containing 0.075 – 0.15 units/ml of Xylanase in cold deionized water.)
- D. 500 mM Sodium Hydroxide Solution
(Prepare 200 ml using deionized water and Sodium Hydroxide (1.0 N) Sigma Stock No. 930-65.)
- E. 0.5% (w/v) p-Hydroxybenzoic Acid Hydrazide Solution (PAHBAH)
(Prepare 200 ml in Reagent D using p-Hydroxybenzoic Acid Hydrazide, Sigma Prod. No. H-9882. **PREPARE FRESH BEFORE USE.**)
- F. 0.2 mg/ml Glucose Standard Solution (Glucose Std)
(Prepare by diluting Glucose Standard Solution, Sigma Stock No. 14-11, with deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Deionized Water	-----	1.00
Reagent A (Buffer)	3.00	3.00
Reagent B (Xylan)	1.00	1.00

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

Reagent C (Enzyme Solution)	1.00	-----
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Mix by swirling and incubate at 37°C for exactly 60 minutes.

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

	<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>
Deionized water		0.97	0.97	0.95	0.90	0.80	0.60	0.40
Test Solution	0.03	----	----	----	----	----	----	1.00
Blank Solution	----	0.03	----	----	----	----	----	----
Reagent F (Std)	----	----	0.05	0.10	0.20	0.40	0.60	----
Reagent E (PAHBAH)	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00

Immediately mix by swirling and transfer the tubes to a boiling water bath. Incubate for 5 minutes. Remove the tubes from the boiling water bath and allow to cool to room temperature.

Mix by inversion and transfer the solutions to suitable cuvettes. Obtain the A_{410nm} for the Test, Blank, and Standards using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

$$\Delta A_{410nm} \text{ Std} = A_{410nm} \text{ Std} - A_{410nm} \text{ Std Blank}$$

Prepare a standard curve by plotting the ΔA_{410nm} Standard vs the milligrams of Glucose.

Sample Determination:

$$\Delta A_{410nm} \text{ Sample} = A_{410nm} \text{ Test} - A_{410nm} \text{ Blank}$$

Determine the milligrams of glucose using the standard curve.

$$\text{Units/ml enzyme} = \frac{(\text{milligrams of glucose liberated})(5)(df)}{(60) (1) (0.03)}$$

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CALCULATIONS: (continued)

5 = Volume (in milliliters) of assay

df = Dilution factor

60 = Time of assay (in minutes) as per Unit Definition

1 = Volume (in milliliters) of enzyme used

0.03 = Volume (in milliliter) used for reducing sugar 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 from xylan (measured as glucose) per minute at pH 4.5 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) xylan and 0.075 - 0.15 unit xylanase.

REFERENCE:

Chen, W.P., Matsuo, M., and Yasui, T. (1986) *Agric. Biol. Chem.* **50**, 1183-1194

Lever, M. (1972) *Analytical Biochemistry*, Vol. 47, 273-279

NOTES:

1. This assay is based on the cited references.
2. 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.