

Enzymatic Assay of XYLANASE
(EC 3.2.1.8)

PRINCIPLE:

Xylan + H₂O ^{Xylanase} > Reducing Sugar (measured as Xylose)

CONDITIONS: T = 30°C, pH = 4.5, A_{540nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 50 mM Sodium Acetate Buffer, pH 4.5 at 30°C
(Prepare 50 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 4.5 at 30°C with 1 M HCl.)
- B. 1.0% (w/v) Xylan Substrate Solution (Xylan)
(Prepare 5 ml in Reagent A using Xylan, Sigma Prod. No. X-0627.)
- C. 0.05% (w/v) Bovine Serum Albumin (Enz Diluent)
(Prepare 25 ml in Reagent A using Albumin, Bovine, Sigma Prod. No. A-4503.)
- D. Xylanase Enzyme Solution
(Immediately before use, prepare a solution containing 5 - 10 units/ml of Xylanase in cold Reagent C.)
- E. 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.)¹
- F. 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

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REAGENTS: (continued)

G. 1 mg/ml Xylose Standard Solution (Xylose Std)
(Prepare 10 ml in deionized water using D(+)-Xylose,
Sigma Prod. No. X-1500.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std Blank</u>
Reagent B (Xylan)	1.90	1.90	----	----	----	----	----
Reagent G (Xylose Std)	----	----	0.02	0.05	0.07	0.10	----
Reagent C (Enz Diluent)	----	----	1.98	1.95	1.93	1.90	2.00

Mix by swirling and equilibrate to 30°C. Then add:

Reagent D (Enzyme Soln)	0.10	----	----	----	----	----	----
Reagent C (Enz Diluent)	----	0.10	----	----	----	----	----

Mix by swirling and incubate at 30°C for exactly 10 minutes. Then add:

Reagent E (Copper Soln)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
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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 F (Ars-Mol)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
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Shake or vortex the tubes until foaming stops and any precipitate present is dissolved. Centrifuge to clarify.

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

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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 $\Delta A_{540\text{nm}}$ Standard vs the μmoles of Xylose.

Sample Determination:

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

Determine the μmoles of xylose using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of xylose liberated})(\text{df})}{(10)(0.1)}$$

df = Dilution factor

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

0.1 = Volume (in milliliters) 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 1.0 μmole of reducing sugar measured as xylose equivalents from xylan (X-0627) per minute at pH 4.5 at 30°C.

FINAL ASSAY CONCENTRATIONS:

In a 2.00 ml reaction mix, the final concentrations are 50 mM sodium acetate, 0.95% (w/v) xylan, 0.003% (w/v) bovine serum albumin, and 0.5 - 1.0 unit xylanase.

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REFERENCE:

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

NOTES:

1. 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.
2. 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.
3. This assay is based on the cited reference.
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.