Enzymatic Assay of Xylanase
(EC 3.2.1.8)

PRINCIPLE:
Xylan + H₂O → Reducing Sugar (measured as Xylose)

CONDITIONS: T = 30°C, pH = 4.5, A₅₄₀nm, 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)

F. 40 mM Molybdic Acid, 19 mM Arsenic Acid and
   756 mM Sulfuric Acid Solution (Ars-Mol)
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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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B</td>
<td>1.90</td>
<td>1.90</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Reagent G</td>
<td>----</td>
<td>----</td>
<td>0.02</td>
<td>0.05</td>
<td>0.07</td>
<td>0.10</td>
<td>----</td>
</tr>
<tr>
<td>Reagent C</td>
<td>----</td>
<td>----</td>
<td>1.98</td>
<td>1.95</td>
<td>1.93</td>
<td>1.90</td>
<td>2.00</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D</td>
<td>0.10</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Reagent C</td>
<td>----</td>
<td>0.10</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
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<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th></th>
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<th>Blank</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
</tbody>
</table>

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 \( \mu \)moles 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 \( \mu \)moles of xylose using the Standard Curve.

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

\( \text{df} \) = 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 \( \mu \)mole 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.
Enzymatic Assay of XYLANASE  
(EC 3.2.1.8)

REFERENCE:


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.