SIGMA QUALITY CONTROL TEST
PROCEDURE

Enzymatic Assay of XYLANASE Activity in DRISSELASE
(EC 3.2.1.8)
Sigma Prod. No. D-9515

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

CONDITIONS: T = 37°C, pH = 4.5, A₄₁₀nm, 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>-----</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Reagent B (Xylan)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

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

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Reagent C (Enzyme Solution)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

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

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

<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 5</th>
<th>Std Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized water</td>
<td>0.97</td>
<td>0.97</td>
<td>0.95</td>
<td>0.90</td>
<td>0.80</td>
<td>0.60</td>
<td>0.40</td>
<td>1.00</td>
</tr>
<tr>
<td>Test Solution</td>
<td>0.03</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Blank Solution</td>
<td>-----</td>
<td>0.03</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Reagent F (Std)</td>
<td>-----</td>
<td>-----</td>
<td>0.05</td>
<td>0.10</td>
<td>0.20</td>
<td>0.40</td>
<td>0.60</td>
<td>-----</td>
</tr>
<tr>
<td>Reagent E (PAHBAH)</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
</tr>
</tbody>
</table>

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 $\Delta A_{410nm}$ 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

\[
\text{Units/mL enzyme} = \frac{\text{units/mL enzyme}}{\text{mg solid/mL enzyme}}
\]

\[
\text{Units/mg solid} = \frac{\text{units/mL enzyme}}{\text{mg solid/mL enzyme}}
\]

\[
\text{Units/mg protein} = \frac{\text{units/mL enzyme}}{\text{mg protein/mL enzyme}}
\]

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:


NOTES:

1. This assay is based on the cited references.

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

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