SIGMA QUALITY CONTROL TEST PROCEDURE
Enzymatic Assay of CELLULASE
(EC 3.2.1.4)

PRINCIPLE:

Cellulose + H_2O → D-Glucose

D-Glucose + ATP → D-Glucose 6-Phosphate + ADP

D-Glucose 6-Phosphate + β-NAD → 6-PG + β-NADH

Abbreviations used:
ATP = Adenosine 5'-Triphosphate
ADP = Adenosine 5'-Diphosphate
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form
G-6PDH = Glucose 6-Phosphate Dehydrogenase
G-PG = 6-Phospho-D-Gluconate

CONDITIONS:  T = 37°C, pH = 5.0, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 50 mM Sodium Acetate Buffer, pH 5.0 at 37°C
(Prepare 200 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.0 at 37°C with 1 M HCl.)

B. 5% (w/v) Sigmacell Solution (Sigmacell)
(Prepare 100 ml in Reagent A using Cellulose (Sigmacell), Type 20, Sigma Prod. No. S-3504. Mix and heat gently to make a uniform suspension.)

C. Cellulase Enzyme Solution (Cellulase)
(Immediately before use, prepare a solution containing 2 - 6 units/ml of Cellulase in cold deionized water.)

D. Glucose (HK) Determination Vial (16-10)
(Use Glucose (HK) 10, Sigma Stock No. 16-10. Dissolve the contents in 10 ml of deionized water.)
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PROCEDURE:

Step 1:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (Sigmacell)</td>
<td>4.00</td>
<td>4.00</td>
</tr>
</tbody>
</table>

Equilibrate to 37°C. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Cellulase)</td>
<td>1.00</td>
<td>-----</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>-----</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Immediately mix by swirling and incubate at 37°C for exactly 120 minutes with moderate shaking.

Immediately transfer into an ice bath. Allow to stand until the suspension is settled. Centrifuge for 2 minutes to clarify. Use the supernatant in Step 2.

Step 2:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (16-10)</td>
<td>3.00</td>
<td>3.00</td>
</tr>
</tbody>
</table>

Equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Record the initial A_{340nm} for both the Test and Blank. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Supernatant (Step 1)</td>
<td>0.10</td>
<td>-----</td>
</tr>
<tr>
<td>Blank Supernatant (Step 1)</td>
<td>-----</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in A_{340nm} until complete (for approximately 5 minutes). Obtain the final A_{340nm} for both the Test and Blank.

CALCULATIONS:

\[ \Delta A_{340nm} \text{ Test} = A_{340nm} \text{ Test Final} - A_{340nm} \text{ Test Initial} \]

\[ \Delta A_{340nm} \text{ Blank} = A_{340nm} \text{ Blank Final} - A_{340nm} \text{ Blank Initial} \]
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CALCULATIONS: (continued)

\[
\text{Units/ml enzyme} = \frac{(\Delta A_{340nm \text{ Test}} - \Delta A_{340nm \text{ Blank}})(3.1)(5)(df)}{(6.22)(2)(1)(0.1)}
\]

3.1 = Final volume (in milliliters) of Step 2  
5 = Total volume (in milliliters) of reaction mix (Step 1)  
df = Dilution factor  
6.22 = Millimolar extinction coefficient of β-NADH at 340nm  
2 = Conversion factor from 2 hours to 1 hour as per the Unit Definition  
1 = Volume (in milliliter) of cellulase used in Step 1  
0.1 = Volume (in milliliter) from Step 1 used in Step 2

Units/mg solid = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}

UNIT DEFINITION:

One unit will liberate 1.0 µmole of glucose from cellulose in one hour at pH 5.0 at 37°C (2 hour incubation time).

FINAL ASSAY CONCENTRATION:

In a 5.00 ml reaction mix, the final concentrations are 40 mM sodium acetate, 4% (w/v) Sigmacell and 2 - 6 units of cellulase.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.

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

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