Enzymatic Assay of CERAMIDE GLYCANASE

PRINCIPLE:
Monosialoganglioside Ceramide Glycanase > Monosaccharides
Monosaccharides Park-Johnson Reaction → Green Colored Dye Complex

CONDITIONS:  T = 37°C, pH 5.0, A₆₉₀nm, Light path = 1 cm

METHOD:  Colorimetric

REAGENTS:

A.  50 mM Sodium Acetate Buffer, pH 6.0 at 37°C (Buffer) (Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate. Adjust to pH 6.0 at 37°C with 2 M Acetic Acid.)

B.  50 mM Sodium Acetate Buffer with 2.3 mM Sodium Cholate, pH 5.0 at 37°C (Prepare 25 ml in deionized water using Sodium Acetate, Trihydrate, and Cholic Acid, Sodium Salt, Hydrate. Adjust to pH 5.0 at 37°C with 2 M Acetic Acid.)

C.  50% (v/v) Chloroform and 50% (v/v) Methanol Solution (Prepare 5 ml using Chloroform, and Methanol.)

D.  0.047% (w/v) Monosialoganglioside Solution (GM₁) (Prepare by dissolving 1 mg of Monosialoganglioside (GM₁) from Bovine Brain, in 2.15 ml of Reagent C. Evaporate the solution under nitrogen gas. This should be done under a vacuum hood. Dissolve the residue with 2.15 ml of Reagent B and sonicate (4 x 30 sec) with pauses to adjust the temperature of the solution to 4°C. Store at 4°C.)

E.  50 mM Sodium Carbonate and 10 mM Potassium Cyanide Solution (Prepare 100 ml in deionized water using Sodium Carbonate, Anhydrous, and Potassium Cyanide. Caution: Potassium Cyanide is TOXIC.)
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REAGENTS: (continued)

F. 1.5 mM Potassium Ferricyanide Solution (Pot Ferr)  
(Prepare 100 ml in deionized water using Potassium Ferricyanide.)

G. 50 mM Sulfuric Acid Solution  
(Prepare 200 ml in deionized water using Sulfuric Acid.)

H. 3.1 mM Ferric Ammonium Sulfate and 3.5 mM Sodium Dodecyl Sulfate Solution  
(Prepare 200 ml in Reagent G using Ferric Ammonium Sulfate, Dodecahydrate and Lauryl Sulfate, Sodium Salt.)

I. 1.11 mM Glucose Standard Solution (Std)  
(Prepare 1 ml in deionized water using Glucose Standard Solution, 1000 mg/dl.)

J. Ceramide Glycanase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.007 – 0.014 unit/ml of Ceramide Glycanase in cold deionized water.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Std</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (GM₃)</td>
<td>0.05</td>
<td>----</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent I (Std)</td>
<td>----</td>
<td>0.01</td>
<td>----</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.03</td>
<td>0.09</td>
<td>0.05</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Std</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent J (Enzyme Soln)</td>
<td>0.02</td>
<td>----</td>
<td>-----</td>
</tr>
</tbody>
</table>

Immediately mix by swirling and incubate at 37°C for exactly 30 minutes. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Std</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent E</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>Reagent F (Pot Ferr)</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
</tr>
</tbody>
</table>
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PROCEDURE: (continued)

Mix by swirling and incubate at 100°C for 15 minutes in a boiling water bath. Remove from the boiling water bath and equilibrate to room temperature. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Std</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td>Reagent H</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Mix by swirling and incubate at 25°C for 15 minutes. If necessary, clarify the solutions by filtering through a 0.45 µM Millipore filter. Transfer to suitable cuvettes and record the A_{690nm} for the Test, Standard, and Blank.

CALCULATIONS:

Standard:

\[ \Delta A_{690nm} \text{ Standard} = A_{690nm} \text{ Standard} - A_{690nm} \text{ Blank} \]

Sample Determination:

\[ \Delta A_{690nm} \text{ Test} = A_{690nm} \text{ Test} - A_{690nm} \text{ Blank} \]

Determine the µmoles of monosialoganglioside hydrolyzed by comparing it to the standard.

\[ (\mu\text{moles of monosialoganglioside hydrolyzed}) \times \text{(df)} \]

Units/ml enzyme = \[ \frac{(30)(0.02)}{\text{df}} \]

df = Dilution factor
0.02 = Volume (in milliliter) of enzyme used
30 = Time (in minutes) of assay as per the Unit Definition

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 micromole of monosialoganglioside GM₁ per minute at pH 5.0 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 0.10 ml reaction mix, the final concentrations are 0.024% (w/v) monosialoganglioside, 25 mM sodium acetate, 1.2 mM sodium cholate, and 0.00014 - 0.00028 unit ceramide glycanase.
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REFERENCE:

Park, J.T. and Johnson, M.J. (1949) Journal of Biological Chemistry 181, 149-151

NOTES:

1. This assay is based on the cited reference.

2. Where Products are specified, equivalent reagents may be substituted.

This procedure is for informational purposes.