Enzymatic Assay of CERAMIDE GLYCANASE

PRINCIPLE:
Monosialoganglioside \text{\textit{Ceramide Glycanase}} \rightarrow \text{Monosaccharides}
Monosaccharides \text{\textit{Pank-Johnson Reaction}} \rightarrow \text{Green Colored Dye Complex}

CONDITIONS: \( T = 37^\circ C, \text{pH 5.0, } A_{690nm}, \text{Light path = 1 cm} \)

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, Sigma Prod. No. S-8625. 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, Sigma Prod. No. S-8625, and Cholic Acid, Sodium Salt, Hydrate, Sigma Prod. No. C-1254. 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, Sigma Stock No. 27,063-6, and Methanol, Sigma Prod. No. M-3641.)

D. 0.047% (w/v) Monosialoganglioside Solution (GM₃)  
(Prepare by dissolving 1 mg of Monosialoganglioside (GM₃) from Bovine Brain, Boehringer Mannheim, Catalog No. 1087-126, 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, Sigma Prod. No. S-2127, and Potassium Cyanide, Sigma Stock No. 20,781-0. 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, Sigma Prod. No. P-8131.)

G.  50 mM Sulfuric Acid Solution
    (Prepare 200 ml in deionized water using Sulfuric Acid, Sigma Prod. No. S-1526.)

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, Sigma Prod. No. F-3629 and Lauryl Sulfate, Sodium Salt, Sigma Prod. No. L-5750.)

I.  1.11 mM Glucose Standard Solution (Std)
    (Prepare 1 ml in deionized water using Glucose Standard Solution, 1000 mg/dl, Sigma Stock No. 14-11.)

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>
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</thead>
<tbody>
<tr>
<td>Reagent D (GM1)</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>
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</thead>
<tbody>
<tr>
<td>Reagent J (Enzyme Soln)</td>
<td>0.02</td>
<td>----</td>
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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>
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</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>
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</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.

$$\frac{\text{(µmoles of monosialoganglioside hydrolyzed})(df)}{(30)(0.02)}$$

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

Units/ml enzyme =

Units/mg protein =

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 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.