

Enzymatic Assay of CERAMIDE GLYCANASE

PRINCIPLE:

Monosialoganglioside $\xrightarrow{\text{Ceramide Glycanase}}$ Monosaccharides

Monosaccharides $\xrightarrow{\text{Park-Johnson Reaction}}$ ¹ Green Colored Dye Complex

CONDITIONS: T = 37°C, pH 5.0, A_{690nm}, 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 (GM₁)
(Prepare by dissolving 1 mg of Monosialoganglioside (GM₁) 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.)

Enzymatic Assay of CERAMIDE GLYCANASE

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:

	<u>Test</u>	<u>Std</u>	<u>Blank</u>
Reagent D (GM ₁)	0.05	-----	0.05
Reagent I (Std)	-----	0.01	-----
Deionized Water	0.03	0.09	0.05

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

Reagent J (Enzyme Soln)	0.02	----- -----
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Immediately mix by swirling and incubate at 37°C for exactly 30 minutes. Then add:

Reagent A (Buffer)	0.20	0.20	0.20
Reagent E	0.125	0.125	0.125
Reagent F (Pot Ferr)	0.125	0.125	0.125

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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:

	<u>Test</u>	<u>Std</u>	<u>Blank</u>
Reagent H	1.00	1.00	1.00

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 $A_{690\text{nm}}$ for the Test, Standard, and Blank.

CALCULATIONS:

Standard:

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

Sample Determination:

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

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

$$(\mu\text{moles of monosialoganglioside hydrolyzed})(\text{df})$$

$$\text{Units/ml enzyme} = \frac{\quad}{(30)(0.02)}$$

df = Dilution factor

0.02 = Volume (in milliliter) of enzyme used

30 = Time (in minutes) of assay as per the Unit Definition

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

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 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.