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Glucosylceramidase Activity Assay Kit (Fluorometric)

05/19

(Catalog # K2003-100; 100 assays; Store at -20°C)

I. Introduction:

Lysosomal glucosylceramidase also known as acid beta-glucosidase (E.C. 3.2.1.45) is a lysosomal membrane protein that cleaves the beta-glucosidic linkage of glycosylceramide (GC). The enzyme plays a central role in the degradation of complex lipids and the turnover of cellular membranes. Through the production of ceramides, it participates in the PKC-activated salvage pathway of ceramide formation. Glucosylceramidase also plays a role in cholesterol metabolism. It may catalyze the glucosylation of cholesterol through a transglucosylation reaction that transfers glucose from glucosylceramide to cholesterol. Deficiency of Glucosylceramidase results in the accumulation of GC in lysosomes. This leads to Gaucher disease, an inherited disorder that affects many organs and tissues. BioVision's Glucosylceramidase Activity Assay Kit provides a simple, rapid way to monitor Glucosylceramidase activity in a wide variety of biological samples. In this kit, Glucosylceramidase cleaves a specific synthetic substrate and releases a fluorophore, which can be easily quantified (Ex/Em=360/445 nm). The assay is specific, sensitive and can detect as low as 0.2 µU of Glucosylceramidase activity in variety of samples.

Glucosylceramidase Substrate $\xrightarrow{\textbf{Glucosylceramidase}}$ Cleaved Substrate + Fluorescent Product (Ex/Em= 360/445 nm)

II. Applications:

- Measurement of Glucosylceramidase activity in various tissues/cells
- Screening for Gaucher Disease

III. Sample Type:

- Tissue Homogenates: Spleen, etc.
- Cell Lysates: MDA-MB-231 Cell Lysates, 3T3 Cell Lysates, etc.

IV. Kit Contents:

Components	K2003-100	Cap Code	Part Number
Glucosylceramidase Assay Buffer	25 ml	NM	K2003-100-1
Glucosylceramidase Stop Buffer	25 ml	WM	K2003-100-2
Glucosylceramidase Substrate	100 µl	Blue	K2003-100-3
4-Methylumbelliferone Standard	35 µl	Yellow	K2003-100-4
Glucosylceramidase Positive Control	1 vial	Green	K2003-100-5

V. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer
- 96-well clear plate with flat bottom; 96-well white plate is preferred
- Dounce Tissue Homogenizer (Cat. #1998)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. Once opened, use within two months.

- **Glucosylceramidase Assay Buffer & Stop Buffer:** Store at either 4 ºC or -20ºC. Bring to room temperature (RT) before use.
- **Glucosylceramidase Substrate:** Light sensitive. Store at -20°C. Thaw at RT.
- **4-Methylumbelliferone Standard (5 mM):** Light sensitive. Store at -20°C. Thaw at RT.
- **Glucosylceramidase Positive Control:** Reconstitute with 100 µl Glucosylceramidase Assay Buffer. Pipet up and down to mix thoroughly. Aliquot and store at -20ºC. Avoid freeze/thaw. Keep on ice while in use. Use within two months.

VII. Glucosylceramidase Activity Assay Protocol:

1. **Sample Preparation: For Tissue and cells:** Homogenize tissue (10~20 mg) or pelleted cells (~1 x 10^7) with 200 µl ice-cold Glucosylceramidase Assay Buffer and keep on ice for 10 min. Centrifuge Samples at 12,000 x *g* and 4°C for 10 min and collect the supernatant. Add 2-20 µl of Sample(s) into well(s) of a 96-well clear plate. Add same volume of Glucosylceramidase Assay Buffer in well(s) designated as Background Control.

   For Positive Control: Prepare a 10 fold dilution of the reconstitute Glucosylceramidase Positive Control (4 µl of reconstituted Glucosylceramidase Positive Control with 36 µl Glucosylceramidase Assay Buffer). Add 4-10 µl Diluted Glucosylceramidase Positive Control into desired well(s).

   Adjust the volume of **Positive Control**, **Sample(s)**, **Background Control** to **40 µl/well** with Glucosylceramidase Assay Buffer.

   Notes:

   a. We suggest trying several Sample(s) amounts per well to ensure that the readings are within the Standard Curve range and the changes of velocity are within the linear range.

   b. Do not store unused diluted Glucosylceramidase Positive Control.

   c. For some Samples, endogenous small molecules may interfere with the results. In this case, remove the interference by filtering the Samples through a 10 kDa cut-off spin column (BioVision # 1997). Centrifuge at 12,000 x *g* and 4ºC for 10 min and discard the filtrate.

Adjust the ultra-concentrate to the original volume using Glucosylceramidase Assay Buffer and repeat this procedure 3-5 times. Use the ultra-concentrate for Sample loading.

2. **Standard Curve Preparation:** Prepare 100 µM 4-Methylumbelliferone Standard (4-MU) solution by adding 2 µl of 5 mM 4-MU to 98 µl Glucosylceramidase Assay Buffer. Further dilute the 100 µM 4-MU Standard solution 5-fold by adding 20 µl of 100 µM 4-MU to 80 µl Glucosylceramidase Assay Buffer to generate 20 µM 4-MU Standard. Add 0, 2, 4, 6, 8, 10 µl of 20 µM (20 pmol/µl) 4-MU Standard into a series of wells to generate 0, 40, 80, 120, 160, 200 pmol of 4-MU per well respectively. Adjust the volumes to **60 µl/well** with Glucosylceramidase Assay Buffer.

   Note: Equilibrate the Glucosylceramidase Assay Buffer to 37ºC before adding to the wells.

3. **Substrate Hydrolysis:** Prepare sufficient volume of 20-fold dilution of Glucosylceramidase Substrate (dilute 4 µl of Glucosylceramidase stock substrate with 76 µl of Glucosylceramidase Assay Buffer), vortex briefly. Add 20 µl of the diluted Glucosylceramidase Substrate to each well containing the Sample(s), Positive Control and Background Control. The total volume in each well including Sample(s), Positive Control and Background Control should be 60 µl. **Mix well and incubate at 37°C for 30 min., avoid light.** After incubation, add 100 µl of Glucosylceramidase Stop Buffer to each well containing Sample(s), Positive Control, Background Control and Standards. Mix well. The final volume in each well should be 160 µl.

   Notes:

   a. Equilibrate the Glucosylceramidase Assay Buffer and Glucosylceramidase Stop Buffer to 37ºC prior to the assay.

   b. 4-MU Standards can be prepared at the end of the incubation time, and measured in end-point mode.

   c. Do not store the unused diluted Glucosylceramidase Substrate.

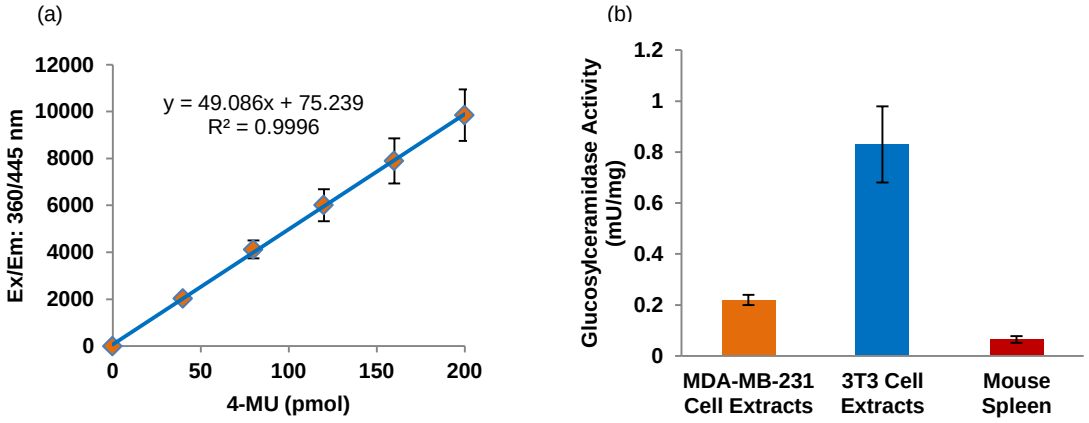
4. **Measurement:** Measure the fluorescence intensity (Ex/Em = 360/445 nm) at 37°C in end point mode.

5. **Calculation:** Subtract 0 Standard reading from all Standard(s) readings. Plot the 4-MU Standard Curve. Subtract the Background Control reading from Sample readings. Apply Sample ΔRFU to 4-MU Standard Curve to obtain the corresponding pmol of product formed (B, in pmol). Calculate the activity of Glucosylceramidase activity in the Sample as:

Specific Sample Glucosylceramidase Activity = B/(30 x V x P) x D = pmol/min.mg ≡ µU/mg

Where: **B** is 4-MU amount from the Standard Curve (pmol)
30 is the Reaction time (min)
V is Sample volume added into the reaction well (ml)
P is Initial Sample Concentration in mg-protein/ml (mg/ml)
D is Sample Dilution Factor

Unit Definition: One unit of Glucosylceramidase activity is the amount of enzyme that generates 1.0 µmol of 4-Methylumbelliferone per min at pH 4.5 at 37°C.



Figures: (a) 4-MU Standard Curve. (b) Measurement of Glucosylceramidase in MDA-MB-231 Cell Lysates (10 µg protein), 3T3 Cell Lysates (5 µg protein) and Mouse Spleen Extracts (5 µg protein). All assays were performed following kit protocol.

VIII. Related Products:

- α-Glucosidase Activity Colorimetric Assay Kit (K690)
- α-Glucosidase Inhibitor Screening Kit (K938)
- Sphingomyelin Quantification Colorimetric Assay Kit (K600)
- Acid Sphingomyelinase Assay Kit II (Colorimetric) (K192)
- Cholesterol Efflux Fluorometric Assay Kit (cell-based) (K582)
- Cholesterol Detection Kit (cell-based) (K587)
- Total cholesterol and cholesteryl Ester Colorimetric/Fluorometric Assay Kit (K623)

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