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ab273339 Glucosylceramidase Activity Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273339
(use https://www.abcam.cn/ab273339 for china, or
https://www.abcam.co.jp/ab273339 for Japan)

For the determination of Glucosylceramidase activity in tissue homogenates and cell lysates.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Glucosylceramidase Activity Assay Kit (Fluorometric) (ab273339) provides a simple, rapid way to monitor Glucosylceramidase (GC) 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.

2. Protocol Summary

Prepare all reagents, samples and positive controls.

Prepare standards.

Add all samples to the appropriate wells.

Prepare and add Glucosylceramidase Substrate to the appropriate wells.

Measure fluorescence at 37°C in end-point mode.

Determine Glucosylceramidase activity using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
4-Methylumbelliferone Standard	35 µl	-20°C
Assay Buffer 25	25 ml	-20°C
Glucosylceramidase Positive Control	1 vial	-20°C
Stop Solution V	25 ml	-20°C
Glucosylceramidase Substrate	100 µl	-20°C

PLEASE NOTE: Assay Buffer 25 was previously labelled as Assay Buffer XXV and Glucosylceramidase Assay Buffer. The composition has not changed.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multi-well spectrophotometer
- 96-well white plate with flat bottom
- Dounce tissue homogenizer

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

– Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer 25:**
Ready to use as supplied. Store at either -20°C. Bring to room temperature before use.

9.2 **Stop Solution V:**
Ready to use as supplied. Store at -20°C. Bring to room temperature before use.

9.3 **4-Methylumbelliferone Standard:**
Light sensitive. Store at -20°C. Thaw at RT.

9.4 **Glucosylceramidase Positive Control:**
Reconstitute with 100 µl Assay Buffer 25. Pipet up and down to mix thoroughly. Aliquot and store at -20°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.

9.5 **Glucosylceramidase Substrate:**
Light sensitive. Store at -20°C. Thaw at RT.

10. Sample Preparation

Tissue/cell lysate preparation:

10.1 Homogenize tissue (10~20 mg) or pelleted cells (~1 x 10^7) with 200 µl ice-cold Assay Buffer 25 and keep on ice for 10 mins.

10.2 Centrifuge Samples at 12,000 x *g* and 4°C for 10 mins and collect the supernatant.

10.3 Add 2-20 µl of Sample(s) into well(s) of a 96-well white clear bottom plate.

10.4 Add same volume of Assay Buffer 25 Buffer in well(s) designated as Background Control.

Δ **Note:** For unknown samples, we suggest testing several doses of your sample to make sure the readings are within the standard curve range.

Δ **Note:** 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. Centrifuge at 12,000 x *g* and 4°C for 10 mins and discard the filtrate. Adjust the ultra-concentrate to the original volume using Assay Buffer 25 and repeat this procedure 3 - 5 times. Use the ultra-concentrate for Sample loading.

11. Standard Curve

11.1 Prepare 100 µM 4-Methylumbelliferone Standard (4-MU) solution by adding 2 µl of 5 mM 4-MU to 98 µl Assay Buffer 25.

11.2 Further dilute the 100 µM 4-MU Standard solution 5-fold by adding 20 µl of 100 µM 4-MU to 80 µl Assay Buffer 25 to generate 20 µM 4-MU Standard.

11.3 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.

11.4 Adjust the volumes to 60 µl/well with Assay Buffer 25.

Standard #	20 µM 4-MU Standard (µL)	Assay Buffer 25 (µL)	4-MU (pmol/well)
1	0	60	0
2	2	58	40
3	4	56	80
4	6	54	120
5	8	52	160
6	10	50	200

Δ Note: Equilibrate the Assay Buffer 25 to 37°C before adding to the wells

12. Assay Procedure

– Keep on ice while in use.

12.1 **Glucosylceramidase Positive Control:** Prepare a 10 fold dilution of the reconstitute Glucosylceramidase Positive Control (4 µl of reconstituted Glucosylceramidase Positive Control with 36 µl Assay Buffer 25).
12.2 Add 4-10 µl Diluted Glucosylceramidase Positive Control into desired well(s).
12.3 Adjust the volume of Positive Control, Sample(s), Background Control to 40 µl/well with Assay Buffer 25.

Δ Note: Do not store un-used diluted Glucosylceramidase Positive Control.

12.4 **Substrate Hydrolysis:** Prepare sufficient volume of 20-fold dilution of Glucosylceramidase Substrate (dilute 4 µl of Glucosylceramidase stock substrate with 76 µl of Assay Buffer 25), vortex briefly.
12.5 Add 20 µl of the diluted Glucosylceramidase Substrate to each well containing the Sample(s), Positive Control and Background Control.
12.6 The total volume in each well including Sample(s), Positive Control and Background Control should be 60 µl.
12.7 Mix well and incubate at 37°C for 30 mins avoid light.
12.8 After incubation, add 100 µl of Stop Solution V to each well containing Sample(s), Positive Control, Background Control and Standards. Mix well.
12.9 The final volume in each well should be 160 µl.

Δ Note: Equilibrate the Assay Buffer 25 and Stop Solution V to 37°C prior to the assay.
Δ Note: 4-MU Standards can be prepared at the end of the incubation time and measured in end-point mode.
Δ Note: Do not store the unused diluted Glucosylceramidase Substrate.

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

13. Calculations

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

$$GC\ activity\ = \frac{B}{(30 \text{ x V x P})}\ X\ D = pmol/min/mg = \mu U/mg$$

B = 4-MU amount from the Standard Curve (pmol)
30 = Reaction time (mins)
V = Sample volume added into the reaction well (ml)
P = Initial Sample Concentration in mg-protein/ml (mg/ml)
D = Dilution factor

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

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

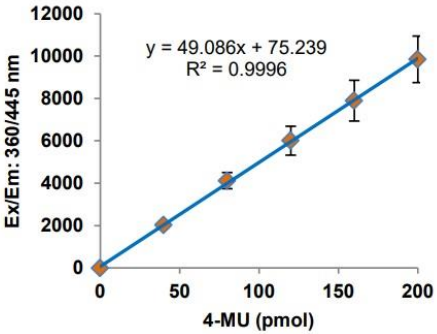

Figure 1. 4-MU Standard Curve.

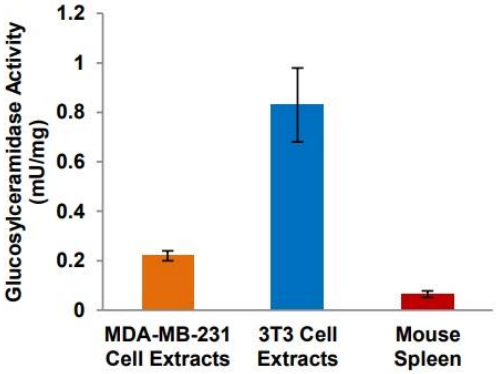

Figure 2. Measurement of Glucosylceramidase in MDA-MB-231 cell lysates (10 µg protein), 3T3 cell lysates (5 µg protein) and mouse spleen extracts (5 µg protein).

15. FAQ / Troubleshooting

General troubleshooting points are found at https://www.abcam.com/en-us/products/biochemical-assays.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us

https://www.abcam.cn/contact-us (China)

https://www.abcam.co.jp/contact-us (Japan)