SensoLyte® Blue Glucocerebrosidase (GBA) Assay Kit *Fluorimetric*

Revision number:1.1	Last updated: April 2019
Catalog #	**AS-72258**
Kit Size	100 Assays (96-well plate)

- ***Optimized Performance:*** This kit is optimized to detect GBA activity.
- ***Enhanced Value:*** Ample reagents to perform 100 assays in a 96-well format.
- ***High Speed:*** The entire process can be completed in one hour.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	GBA substrate Ex/Em=365 nm/445 nm upon cleavage	100 µL
Component B	4MU, reference standard	20 µL
Component C	GBA enzyme, Human Recombinant	10 µL
Component D	Assay Buffer	25 mL
Component E	GBA inhibitor	15 µL
Component F	Stop solution	10 mL

Other Materials Required (but not provided)

- Microplate: Black, flat-bottom, 96-well plate with non-binding surface.
- Fluorescence microplate reader: Capable of detecting emission at 440 ±10 nm with excitation at 365± 10nm.

Storage and Handling

- Store all kit components at -20°C, except for Component C.
- Store Component C at -80°C. Aliquot as needed to avoid freeze-thaw cycles.
- Protect Components A and B from light and from moisture.
- Component D and F can be stored at room temperature for convenience.

Introduction

Glucocerebrosidase (GBA)—also referred to as GCase, acid β-glucosidase, and Glucosylceramidase—is a lysosomal enzyme responsible for the breakdown of glucocerebroside releasing glucose and ceramide. Deficiency of this enzyme due to genetic mutations leads to accumulation of glucocerebroside and development of lysosomal storage disease, known as Gaucher disease (GD).[1] Mutations in the glucocerebrosidase (GBA1) gene are also associated with increased risk for Parkinson disease and related disorders.[2,3] It has been hypothesized that GBA, when not available to clear out proteins like alpha-synuclein, results in the accumulation of the proteins thereby contributing to Parkinson's disease.[4]

The SensoLyte® Blue Glucocerebrosidase (GBA) Assay Kit detects GBA activity by using a common fluorogenic substrate, MUGlc, for measurement of GBA activity. In the presence of GBA the colorless substrate is hydrolyzed into 4-methylumbelliferone (4MU), and after addition of stop solution a bright blue fluorescence can be monitored at excitation/emission = 365nm/445nm.

Protocol

1. Prepare working solutions.

Note: Bring all kit components to room temperature before starting the experiment. Component C should be kept on ice after thawing.

1.1 GBA substrate solution: Dilute GBA substrate (Component A) 50-fold in assay buffer (Component D) according to Table 1. For each experiment, prepare fresh substrate solution.

Table 1. GBA substrate solution for one 96-well plate (100 assays)

Components	Volume
GBA substrate (50X, Component A)	100 µL
1X assay buffer	4.90 mL
Total volume	5 mL

1.2 GBA enzyme solution: Dilute GBA (Component C) 800-fold in assay buffer (Component D) according to Table 2. If not using entire plate, adjust dilution volumes accordingly. The suggested volume of GBA enzyme solution is 40µl/well.

Note 1: Prepare enzyme solution immediately before use. Do not vortex the enzyme solutions. Prolonged storage or vigorous agitation of the diluted enzyme will cause denaturation. Store the enzyme solution on ice.

Table 2. GBA enzyme solution for one 96-well plate (100 assays).

Components	Volume
GBA enzyme (Component C)	5 µL
Assay buffer	3.995 mL
Total volume	4 mL

1.3 Inhibitor (Isofagomine D-tartrate): Dilute the 100 μM inhibitor solution (Component E) 1:100 in assay buffer. The diluted inhibitor solution has a concentration of 1 μM.

2. Set up the enzymatic reaction.

2.1 Add Test compounds and GBA enzyme solution to the microplate wells. For one well of a 96-well plate, the suggested volume of GBA enzyme solution (step 1.2) is 40 μL and 10 μL of test compound.

2.2 Establish the following control wells at the same time, as deemed necessary:

- Positive control: Add 40μl GBA enzyme solution (step 1.2) and 10 μl assay buffer (Component D).
- Inhibitor/Activator control: Add 40μl GBA enzyme solution (step 1.2) and 10 μl GBA inhibitor (step 1.3).
- Vehicle control: Add 40μl GBA enzyme solution (step 1.2) and 10 μl vehicle solution used in delivering the test compound (e.g. DMSO—concentration not to exceed 1%).
- Test compound control: Add 40μl assay buffer (Component D) and 10uL test compound. Some test compounds may themselves be fluorescent and thereby give false results.
- Substrate control: Add 50μl assay buffer (Component D).

2.3 The total volume of all controls should be 50 μL.

3. Detect GBA activity.

3.1 Add 50 μL of the prepared GBA substrate solution (step 1.1) into each well. Mix the reagents completely by shaking the plate gently for no more than 30 sec.

3.2 Incubate the plate for 20 minutes at 37°C.

3.3 Add 100 μL of the Stop solution (Component F) and mix thoroughly.

3.4 Measure fluorescence signal at Ex/Em=365 nm/445 nm

For methods of data analysis: Refer to Appendix I.

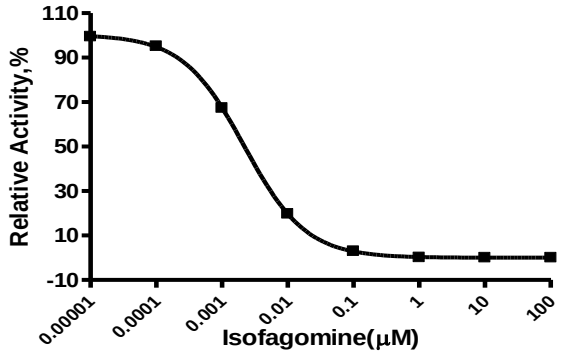

Figure1. Inhibition of GBA activity by Isofagomine as measured with SensoLyte® Blue Glucocerebrosidase Assay Kit.

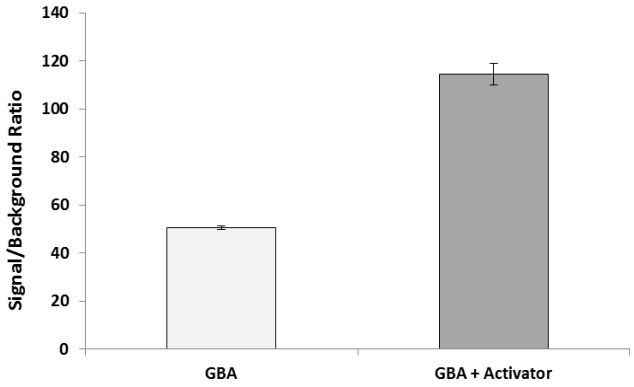

Figure2. Activation of GBA activity by GCase Activator (NCGC00188758). 5ng of GBA enzyme was incubated with and without 10μM of GCase activator and fluorescence was measured with SensoLyte®Blue Glucocerebrosidase Assay Kit.

Appendix I. Data Analysis

- The fluorescence reading from the substrate control well is used as the background fluorescence. This background reading should be subtracted from the readings of the other wells containing substrate. All fluorescence readings are expressed in relative fluorescence units (RFU).
  - Plot data as RFU versus concentration of test compounds.
  - A variety of data analyses can be done, e.g., determining inhibition %, EC_{50}, IC_{50}, etc.

Appendix II: Instrument Calibration

- 4MU reference standard: Dilute the 10mM reference standard (Component B) 50 fold to 200 μM in assay buffer. Perform 2-fold serial dilutions with this diluted standard to obtain 100, 50, 25, 12.5, and 6.25 and 3.126 μM solutions, including an assay buffer blank. Add 50 μL/well of the serially diluted solution.
- Add 50 μL/well of the diluted GBA substrate solution (from step 1.1).
- Incubate the plate for 10 min at 37°C.
- Add 100 μL of the Stop solution (Component F) and mix thoroughly.
- Measure the fluorescence of the reference standard and substrate control wells at Ex/Em=365/445 nm. Use the same setting of sensitivity as used in the enzyme reaction.
- Plot 4MU fluorescence reference standard as RFU (relative fluorescence unit) versus concentration as shown in Figure 3.
- Note: The final concentrations of 4MU reference standard solutions are 50, 25, 12.5, 6.25, 3.125, 1.56, 0.76 and 0 μM. The reference standard is used to calibrate the variation of different instruments and to account for experimental variability. It is also an indicator of the amount of final product of the GBA enzymatic reaction.

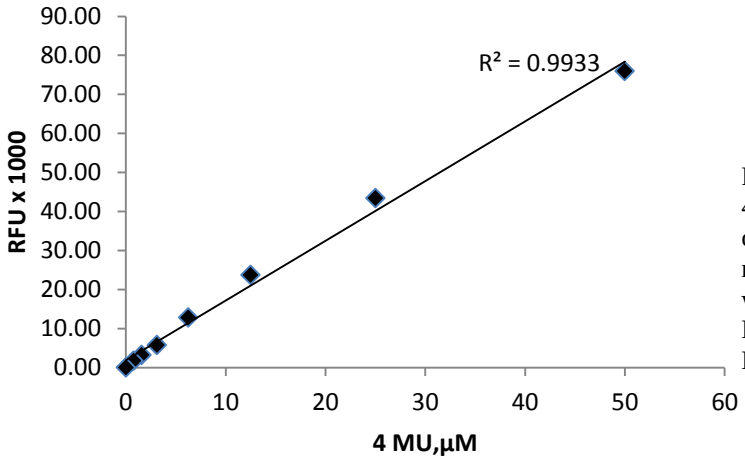

Figure3. 4MU reference standard curve. 4MU was serially diluted in assay buffer, containing GBA substrate and after 10 minutes incubation at 37°C, stop solution was added and fluorescence monitored at Ex/Em= 365 nm/465 nm (SpectraMax M5^e, Molecular Devices).

References:

1. Xu,Y.H et al, *Am.J.Pathol* ,163, 2093(2003)
2. Sidransky, E et al, *N Engl J Med, 361*, 1651(2009)
3. Murphy K.E et al, *Brain*,137, 834(2014)
4. Sybertz, E. et al, *J Lipid Res* 55, 1996 (2014)