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

Revision number:1.1	*Last updated: April 2019*
Catalog #	**AS-72259**
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=570 nm/610 nm upon cleavage	60 µL
Component B	Resorufin, Fluorescence Reference standard , Ex/Em=570 nm/610 nm	10 µL
Component C	GBA enzyme, Human Recombinant	10 µL
Component D	Assay Buffer	25 mL
Component E	GBA inhibitor	15 µL

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 610 ±10 nm with excitation at 570± 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 moisture.
- Component D 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 an 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® Red Glucocerebrosidase (GBA) Assay Kit detects GBA activity using a highly sensitive fluorogenic substrate. This GBA substrate upon Glucocerebrosidase cleavage, releases the red fluorescent dye resorufin, with absorption/emission maxima at 570 nm/610 nm. This assay kit can be used to detect enzyme activity in purified enzyme preparations and for compound screening. The Red Glucocerebrosidase (GBA) Assay is a one-step homogenous reaction, which does not require the additional step of dispensing stop solution after incubation, and is therefore suitable for HTS. The long wavelength fluorescence of resorufin encounters less interference from autofluorescence of components in biological samples and test compounds.

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) 100-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 (100X, Component A)	50 µL
Assay buffer	4.95 mL
Total volume	5mL

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 prepared GBA enzyme solution is 40µl/well.

Note: 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 100μM 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 Measure fluorescence signal at Ex/Em=570±10nm/610 ±10nm

- For kinetic reading: Immediately start measuring fluorescence intensity at Ex/Em=570±10nm/610 ±10nm continuously and record data every 5 min. for 30 to 60 min.
- For end-point reading: Incubate the reaction at room temperature for 30 to 60 min. Keep plate from direct light. Then measure fluorescence intensity at Ex/Em=570±10nm/610 ±10nm.

For methods of data analysis: Refer to Appendix I

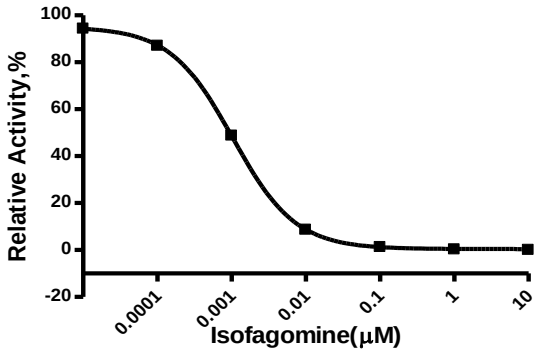

Figure1. Inhibition of GBA activity by Isofagomine as measured with SensoLyte® Red Glucocerebrosidase Assay Kit.

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).

- For kinetics analysis:
  - Plot data as RFU versus time for each sample. To convert RFUs to the concentration of the product of the enzymatic reaction, please refer to Appendix II for establishing a fluorescence reference standard.
  - Determine the range of initial time points during which the reaction is linear. Typically, the first 10-15% of the reaction will be the optimal range.
  - Obtain the initial reaction velocity (V_o) in RFU/min by determining the slope of the linear portion of the data plot.
  - A variety of data analyses can be done, e.g., determining inhibition %, $EC_{50,}$ IC_{50}, K_m, K_i, etc.
- For endpoint analysis:
  - 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

- Resorufin reference standard: Dilute the 4mM reference standard (Component B) 100 fold to 40 µM in assay buffer. Perform 2-fold serial dilutions with this diluted standard to obtain 20, 10, 5, 2.5, 1.25, and 0.625µM 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).
- Measure the fluorescence of the reference standard and substrate control wells at Ex/Em=570±10nm/610 ±10nm. Use the same setting of sensitivity as used in the enzyme reaction.
- Plot Resorufin fluorescence reference standard as RFU (relative fluorescence unit) versus concentration as shown in Figure 2.
- Note: The final concentrations of Resorufin reference standard solutions are 20, 10, 5, 2.5, 1.25, 0.625 and 0.312 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.

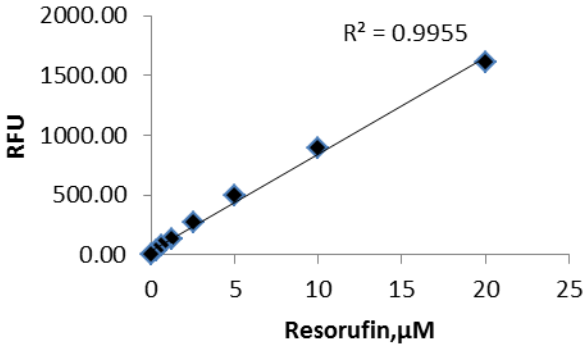

Figure 2. Resorufin reference standard curve. Resorufin was serially diluted in assay buffer containing GBA substrate and fluorescence monitored at Ex/Em= 670 nm/710 nm (SpectraMax M5^e, Molecular Devices).

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)