

**DESCRIPTION**

**Source** Chinese Hamster Ovary cell line, CHO-derived human Glucosylceramidase/GBA protein  
Ala40-Gln536, with a C-terminal 6-His tag  
Accession # P04062.3

**N-terminal Sequence Analysis** Ala40

**Predicted Molecular Mass** 56 kDa

**SPECIFICATIONS**

**SDS-PAGE** 60-75 kDa, reducing conditions

**Activity** Measured by its ability to hydrolyze 4-methylumbelliferyl-β-D-glucopyranoside.  
The specific activity is >200 pmol/min/μg, as measured under the described conditions.

**Endotoxin Level** <1.0 EU per 1 μg of the protein by the LAL method.

**Purity** >95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

**Formulation** Supplied as a 0.2 μm filtered solution in Sodium Citrate. See Certificate of Analysis for details.

**Activity Assay Protocol**

- Materials**
- Assay Buffer: 50 mM Sodium Citrate, 25 mM Sodium Cholate, 5 mM DTT, pH 6.0
  - Stop solution: 0.5 M Glycine, 0.3 M NaOH (~pH 10)
  - Recombinant Human Glucosylceramidase/GBA (rhGBA) (Catalog # 7410-GHB)
  - Substrate: 4-Methylumbelliferyl-β-D-glucopyranoside (Sigma, Catalog # M3633), 10 mM stock in DMSO
  - F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
  - Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

- Assay**
1. Dilute rhGBA to 0.2 ng/μL in Assay Buffer.
  2. Dilute Substrate to 6 mM in Assay Buffer.
  3. Load 25 μL of 0.2 ng/μL rhGBA into wells of a plate, and start the reactions by adding 25 μL of 6 mM Substrate. Include a Substrate Blank containing 25 μL of Assay Buffer and 25 μL of 6 mM Substrate.
  4. Seal plate and incubate at 37 °C for 20 minutes.
  5. After incubation, stop the reactions by adding 50 μL of Stop Solution to each well.
  6. Read at excitation and emission wavelengths of 365 nm and 445 nm (top read), respectively, in endpoint mode.
  7. Calculate specific activity:

$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Adjusted Fluorescence* (RFU)} \times \text{Conversion Factor** (pmol/RFU)}}{\text{Incubation time (min)} \times \text{amount of enzyme (}\mu\text{g)}}$$

\*Adjusted for Substrate Blank

\*\* Derived using calibration standard 4methylumbelliferone (Sigma, Catalog # 69580).

- Final Assay Conditions**
- Per Well:
- rhGBA: 0.005 μg
  - Substrate: 1.5 mM

**PREPARATION AND STORAGE**

**Shipping** The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

- Stability & Storage** Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
- 6 months from date of receipt, -20 to -70 °C as supplied.
  - 3 months, -20 to -70 °C under sterile conditions after opening.

**BACKGROUND**

Glucosylceramidase is a lysosomal enzyme that cleaves the beta-glucosidic linkage of glucosylceramide (1, 2), an intermediate in glycolipid metabolism. The mature enzyme has 497 amino acids with a molecular weight of 62 kDa (3). Glycosylation occurs at four of five N-glycosylation sites and is essential for the trafficking and activity of the enzyme (4). The enzyme is activated in lysosomes by saposin C, although the mechanism of activation is not well understood (5). Defects in Glucosylceramidase are the cause of Gaucher disease, also known as glucocerebrosidase deficiency (6). Gaucher disease is the most prevalent lysosomal storage disease, characterized by accumulation of glucosylceramide in the reticulo-endothelial system. Symptoms of Gaucher disease may include enlarged spleen and liver, liver malfunction, skeletal disorders and bone lesions, severe neurologic complications, swelling of lymph nodes, anemia, low blood platelets and yellow fatty deposits on the white of the eye (7). Currently, enzyme replacement therapy is used to treat patients with the disease (8, 9).

**References:**

1. Sorge, J. *et al.* (1985) *Proc. Natl. Acad. Sci. USA* **82**:7289.
2. Ginns, E. I. *et al.* (1984) *Biochem. Biophys. Res. Commun.* **123**:574.
3. Horowitz, M. *et al.* (1989) *Genomics* **4**:87.
4. Grace, M.E. *et al.* (1994) *J. Biol. Chem.* **269**:2283.
5. Bruhn, h. (2005) *Biochem. J.* **389**:249.
6. Liou, B. *et al.* (2006) *J. Biol. Chem.* **281**:4242.
7. Grabowski, G.A. (2008). *Lancet* **372**:1263.
8. Zheng, W. *et al.* (2007) *Proc. Natl. Acad. Sci. USA* **104**:13192.
9. Beutler, E. and Gelbart, T. (1996) *Hum. Mutat.* **8**:207.