Recombinant Human α-Galactosidase A/GLA

Catalog Number: 6146-GH

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human alpha-Galactosidase A/GLA protein Met1-Leu429, with a C-terminal 6-His tag Accession # P06280
N-terminal Sequence Analysis	Leu32
Predicted Molecular Mass	46 kDa

SPECIFICATIONS

SDS-PAGE	45-55 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze 4-methylumbelliferyl-α-D-galactopyranoside. The specific activity is >1,100 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 Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Sodium Citrate, 50 mM NaCl, pH 4.0
- Recombinant Human α-Galactosidase A/GLA (rhGLA) (Catalog # 6146-GH)
- Substrate: 4-methylumbelliferyl-α-D-galactopyranoside (Sigma, Catalog # M7633), 6.7 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 rhGLA to 4.0 ng/µL in Assay Buffer.
2. Dilute Substrate to 800 µM in Assay Buffer.
3. Load into plate 50 µL of 4.0 ng/µL rhGLA, and start the reaction by adding 50 µL of 800 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL Substrate.
4. Read at excitation and emission wavelengths of 365 nm and 445 nm, respectively, in kinetic mode for 5 minutes.
5. Calculate the specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard 4-Methylumbelliferone (Sigma, Catalog # M1381).

Final Assay Conditions

Per Well:

- rhGLA: 0.200 µg
- Substrate: 400 µM

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

Human α-Galactosidase A is a homodimeric glycoprotein that can release terminal α-galactosyl moieties from glycolipids and glycoproteins and catalyze the hydrolysis of melibiose into galactose and glucose (1). It is a lysosomal enzyme and is responsible for degradation of glycolipid globotriaosylceramide (Gb3) (Galα1-4Galβ1-4Glcβ-ceramide). Mutations in this gene cause Fabry disease, an X-linked hereditary lysosomal storage disease with the accumulation of Gb3 in the walls of small blood vessels, nerves, dorsal root ganglia, renal glomerular and tubular epithelial cells, and cardiomyocytes (2, 3). Inability to prevent the glycosphingolipid deposition can cause hypertension, strokes, heart attack and progressive renal failure (4). Current treatment for Fabry disease is enzyme replacement therapy using intravenously delivered recombinant α-Galactosidase A (5, 6).

References:

1. Ioannou, Y.A. *et al.* (1998) Biochem. J. **332**:789.
2. Koide, T. *et al.* (1990) FEBS Lett. **259**:353.
3. Ioannou Y.A, *et al.* (1992) J. Cell Biol. **119**:1137.
4. Germain, D.P. (2002) Expert. Opin. Investig. Drugs. **11**:1467.
5. Barngrover, D. (2003) J. Biotechnol. **95**:280.
6. Mignani, R. and Cagnoli, L. (2004) J. Nephrol. **17**:354.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449