bio-techne®
R&D SYSTEMS

Recombinant Mouse Lysosomal α-Glucosidase His-tag

Catalog Number: 11400-GH

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived mouse Lysosomal alpha-Glucosidase protein Glu70-Ser953 with a N-terminal 6-His tag Accession # P70699.2
N-terminal Sequence Analysis	His
Predicted Molecular Mass	99 kDa

SPECIFICATIONS

SDS-PAGE	97-107 kDa, under reducing conditions
Activity	Measured by its ability to release glucose from starch. The specific activity is >5000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 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: 0.1 M Sodium Acetate, pH 4.5
- Recombinant Mouse Lysosomal alpha-Glucosidase/GAA (rmGAA) (Catalog # 11400-GH)
- Substrate: Starch from potato, 2% (w/v) stock in deionized water
- Stop Solution: 4.4 mM Dinitrosalicylic Acid, 1 M Potassium Tartrate, 0.4 M Sodium Hydroxide in deionized water
- Maltose Standard, 20 mM stock in deionized water
- 96 well Clear Plate (Catalog # DY990)
- Plate Reader with Absorbance Read Capability

Assay

1. Dilute 20 mM Maltose standard by adding 200 µL of 20 mM Maltose Standard to 600 µL of Assay Buffer for a 5 mM stock. This is the first point of the standard curve.
2. Prepare the standard curve by performing five one-half serial dilutions of the 5 mM Maltose stock in Assay Buffer. Make sure there are 400 µL in each tube for each point of the curve (remove 400 µL from the last point of the curve). Prepare one tube with only 400 µL of Assay Buffer for the curve blank. The standard curve has a range of 19.5 to 625 nmol per well.
3. Dilute rmGAA to 32 µg/mL in Assay Buffer.
4. Dilute 2% starch to 1.5% in Assay Buffer.
5. Prepare reactions by combining 20 µL of diluted rmGAA with 380 µL of 1.5% starch (step 4). Include a control by combining 20 µL of Assay Buffer with 380 µL of 1.5% starch.
6. Vortex, spin, and then incubate reactions, control, and standard curve at 37 °C for 1 hour.
7. Add 400 µL of Stop Solution to all vials, including standard curve.
8. Heat all vials at 95-100 °C for 6 minutes. Then, cool on ice. Tip: Use lid-locks to keep vials closed when heating.
9. Load 250 µL of each dilution of the standard curve, reactions, and controls to empty wells in clear plate.
10. Read plate at 546 nm (absorbance) in endpoint mode.
11. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted glucose produced* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the maltose standard curve using linear or 4-parameter fitting and adjusted for Control.

Final Assay Conditions

Per Well

- rmGAA: 0.2 µg
- Starch: 0.71%

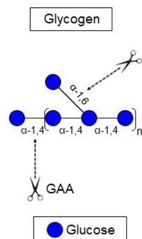
Bio-Techne®
Global | bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL: 1.612.379.2956
USA | TEL: 800.343.7475 Canada | TEL: 855.668.8722 Europe | Middle East | Africa TEL: +44.0.1235.529449
China | info.cn@bio-techne.com TEL: 400.821.3475

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, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

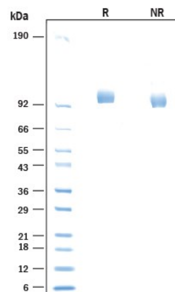
DATA

Enzyme Activity



Recombinant Mouse Lysosomal α-Glucosidase His-tag Protein Enzyme Activity Diagram. Recombinant Mouse Lysosomal α-Glucosidase His-tag Protein, CF (Catalog # 11400-GH) hydrolyses both alpha-1,4- and alpha-1,6-glucosidic linkages on glycogen to release terminal glucose.

SDS-PAGE



Recombinant Mouse Lysosomal α-Glucosidase His-tag Protein SDS-PAGE. 2 μg/lane of Recombinant Mouse Lysosomal α-Glucosidase His-tag Protein (Catalog # 11400-GH) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 97-107 kDa, under reducing conditions.

BACKGROUND

Acid alpha-glucosidase (GAA) is an essential enzyme for the hydrolysis of glycogen α1-4 and α1,6-glycosidic linkages within the lysosome (1,2). GAA is a member of the glycoside hydrolase family GH31 and contains an N-terminal trefoil-P domain, a β-sheet domain, a catalytic barrel, and two C-terminal β-sheet domains (2). In addition to an active site and substrate binding domain, GAA has an additional reported secondary substrate-binding domain that may enhance the processivity of the enzyme (2). Mouse GAA has approximately 80% homology with human GAA. Defects in GAA cause glycogen storage disease II, also known as Pompe's disease, which is a rare autosomal recessive metabolic disorder that damages muscle and nerve cells due to accumulation of glycogen in the lysosome (3). Pompe disease occurs in babies, children, and adults who inherit a defective GAA gene and affects an estimated 5,000 to 10,000 people worldwide (4). Enzyme replacement therapy (ERT) is used to treat patients with Pompe disease and other lysosomal storage diseases (LSDs) (5, 6). Alternative therapeutic strategies such as pharmacological chaperone therapy (PCT) are being explored for use in concert with or independently for the potential to stabilize the target enzyme without impact to the catalytic activity (2, 7, 8).

References:

1. Hoefsloot, L.H. *et. al.* (1988) EMBO J. **7**:1697.
2. Roig-Zamboni, V. *et. al.* (2017) Nat. Commun. **8**:1111.
3. Wan, L. *et. al.* (2008) J. Neurol. **255**:831.
4. Fukuda, T. *et. al.* (2007) Curr. Neurol. Neurosci. Rep. **7**:71.
5. Van Gelder, C.M. *et. al.* (2014) J. Inherit. Metab. Dis. In press.
6. Toscano, A. and B. Schoser. (2013) J. Neurol. **260**:951.
7. Porto, C. *et. al.* (2012) Mol. Ther. **20**:2201.
8. Parenti, G. *et. al.* (2015). Mol. Ther. **23**:1138.

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 Europe | Middle East | Africa TEL: +44.0.1235.529449
China | info.cn@bio-techne.com TEL: 400.821.3475