Product Manual

Beta Hexosaminidase Activity Assay Kit

Catalog Number

MET-5095 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Hexosaminidase is an enzyme that hydrolyzes terminal N-acetyl-D-hexosamine residues from N-acetyl-β-D-hexosaminides. The enzyme, localized to cellular lysosomes, is a dimer formed from either an α or ß subunit which produces three possible dimer combinations. The αß combination (isozyme A) is the only combination with a known function: hydrolyzing GM2 ganglioside in vivo. The other two combinations of αα (isozyme B) and ßß (isozyme S) have been observed in cellular tissues but their function is unknown. Isozyme A works in combination with cofactor GM2 activator protein to degrade GM2 gangliosides and other molecules that have terminal N-acetyl hexosamines. Gene mutations that code for the ß subunit can result in Sandhoff disease. Mutations in the locus for the α subunit decrease the hydrolysis of GM2 gangliosides, which is the primary cause of Tay-Sachs disease. While both the α and ß subunits of lysosomal hexosaminidase can cleave GalNAc residues, only the α subunit is able to hydrolyze GM2 gangliosides because of a key residue, Arg-424, and a loop structure that forms from the amino acid sequence in the alpha subunit. The GM2 activator protein acts as a transporter to localize GM2 gangliosides to hexosaminidase, so a functional hexosaminidase enzyme is able to hydrolyze GM2 gangliosides into GM3 gangliosides.

Cell Biolabs' Beta Hexosamindase Activity Assay Kit is a simple fluorometric assay that measures beta hexosaminidase activity in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, beta hexosaminidase positive control and unknown samples.

Related Products

1. MET-5009: Lipid Extraction & Polar/Neutral Lipid Separation Kit
2. MET-5009-C: Lipid Extraction & Polar/Neutral Lipid Separation Combo Kit
3. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
4. STA-384: Total Cholesterol Assay Kit (Colorimetric)
5. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
6. STA-398: Free Glycerol Assay Kit (Colorimetric)
7. STA-618: Free Fatty Acid Assay Kit (Colorimetric)
8. STA-600: Phosphatidylcholine Assay Kit
9. STA-601: Sphingomyelin Assay Kit
10. STA-613: Lipid Quantification Kit (Colorimetric)

Kit Components

1. Recombinant Beta Hexosaminidase (Part No. 50951D): One 25 µL vial of a 50 µg/mL Recombinant Human Beta Hexosaminidase.
2. 10X Substrate (Part No. 50952C): One 500 µL vial.
3. 5X Assay Buffer (Part No. 50953A): One 30 mL bottle.
4. 10X Neutralization Buffer (Part No. 50954A): One 30 mL bottle.

Materials Not Supplied

1. 96 well black plate
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate Fluorometer

Storage

Upon receipt, store the Recombinant Beta Hexosaminidase and 10X Substrate at -80ºC. Store the remaining components at room temperature.

Preparation of Reagents

- 1X Assay Buffer: Dilute the stock 5X Assay Buffer 1:5 with deionized water for a 1X solution. Stir or vortex to homogeneity. Store unused 1X Assay Buffer at room temperature.
- 1X Substrate: Dilute the 10X Substrate 1:10 with 1X Assay Buffer. For example, add 5 µL of 10X Substrate to 45 µL of 1X Assay Buffer for each well used.

  Note: Prepare only enough 1X Substrate for immediate use.
- 1X Neutralization Buffer: Dilute the stock 10X Neutralization Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity. Store unused 1X Neutralization Buffer at room temperature.

Assay Protocol

1. Add 50 µL of Beta Hexosaminidase samples to the 96-well microtiter black plate.
2. Add 50 µL of the 1X Substrate to each well.
3. Incubate at 37°C for 15 minutes protected from light.
4. Add 100 µL of the 1X Neutralization Buffer to each well.
5. Read the plate at an excitation wavelength of 365 nm and an emission wavelength 450 nm using a microplate fluorometer.

Example of Results

The following figures demonstrate typical Beta Hexosaminidase Activity Assay results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

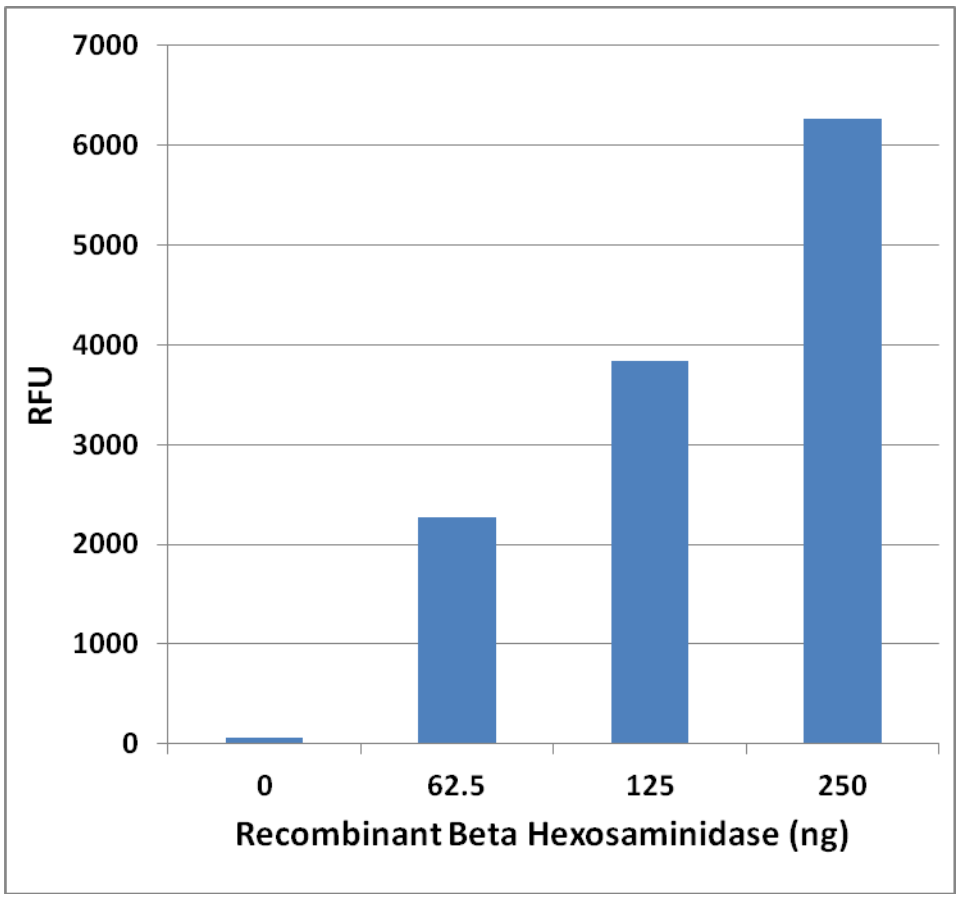

Figure 1: Recombinant Beta Hexosaminidase Positive Control. Various concentrations of the positive control were measured in the Beta Hexosaminidase Activity Assay.

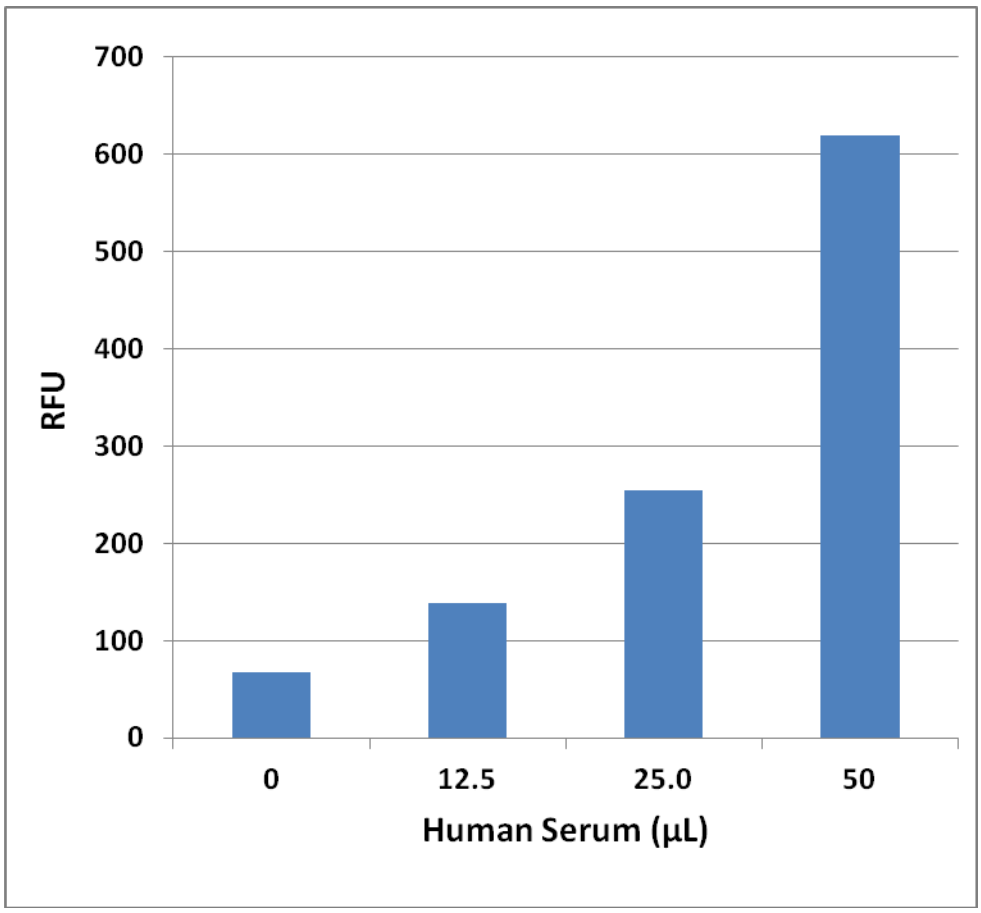

Figure 2: Beta Hexosaminidase Detection in Serum. Human serum was tested according to the Assay Protocol.

References

1. Hou Y, Tse R, Mahuran DJ (1996). *Biochemistry*. **35**: 3963–9.
2. Knapp S, Vocadlo D, Gao Z, Kirk B, Lou J, Withers SG (1996). *J. Am. Chem. Soc.* **118**: 6804–6805.
3. Mark BL, Mahuran DJ, Cherney MM, Zhao D, Knapp S, James MN (2003). *J. Mol. Biol*. **327**: 1093–109.
4. Lemieux MJ, Mark BL, Cherney MM, Withers SG, Mahuran DJ, James MN (2006). *J. Mol. Biol.* **359**: 913–29.

Warranty

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