Product Manual

Beta Hexosaminidase Activity Assay Kit

Catalog Number

MET-5095 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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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 S) and ßß (isozyme B) 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. STA-384: Total Cholesterol Assay Kit (Colorimetric)
2. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
3. STA-618: Free Fatty Acid Assay Kit (Colorimetric)
4. STA-600: Phosphatidylcholine Assay Kit
5. STA-601: Sphingomyelin Assay Kit

Kit Components (shipped on blue ice)

1. Recombinant Beta Hexosaminidase (Part No. 50951D): One 25 µL vial of a 50 µg/mL Recombinant Human Beta Hexosaminidase, for use as optional positive control.
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. 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.
- Beta Hexosaminidase Positive Control: Just prior to use, dilute the provided Recombinant Beta Hexosaminidase (50 µg/mL) 1:10 to 5 µg/mL with 1X Assay Buffer. For example, add 5 µL of the Recombinant Beta Hexosaminidase to 45 µL of 1X Assay Buffer.
- 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.*
  - *10X Substrate is not compatible with polycarbonate (PC), polystyrene (PS), PVC, PET (Polyethylene Terephthalate) or acrylic tubes, vials, pipettes, or pipette tips. It will rapidly degrade or dissolve these materials. Only use glass, PTFE, HDPE, LDPE, PPCO, polycarbonate, or polypropylene (PP) containers, pipettes, or pipette tips.*
- 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 and Beta Hexosaminidase Positive Control (optional) to the 96-well microtiter black plate.

   Note: Samples may be diluted as needed in 1X Assay Buffer.
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.

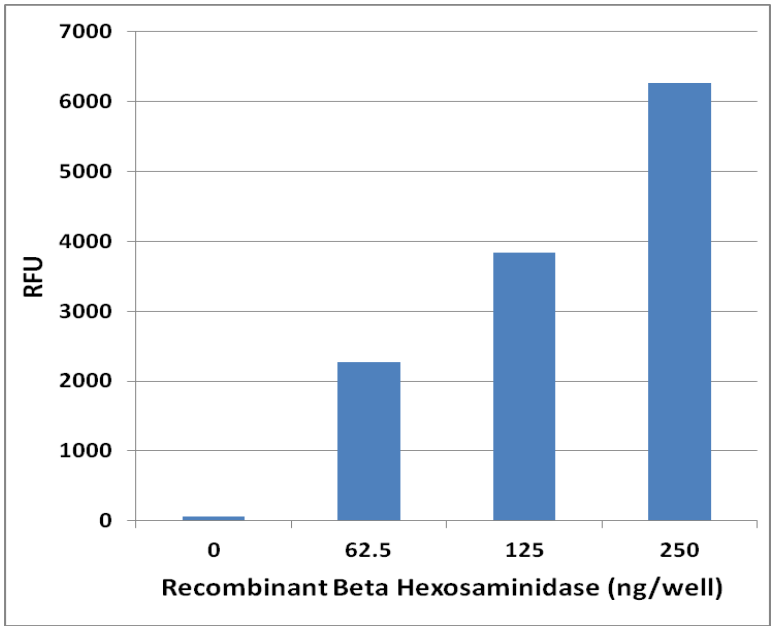


Figure 1: Recombinant Beta Hexosaminidase Positive Control. Various concentrations of the positive control were measured in the Beta Hexosaminidase Activity Assay.

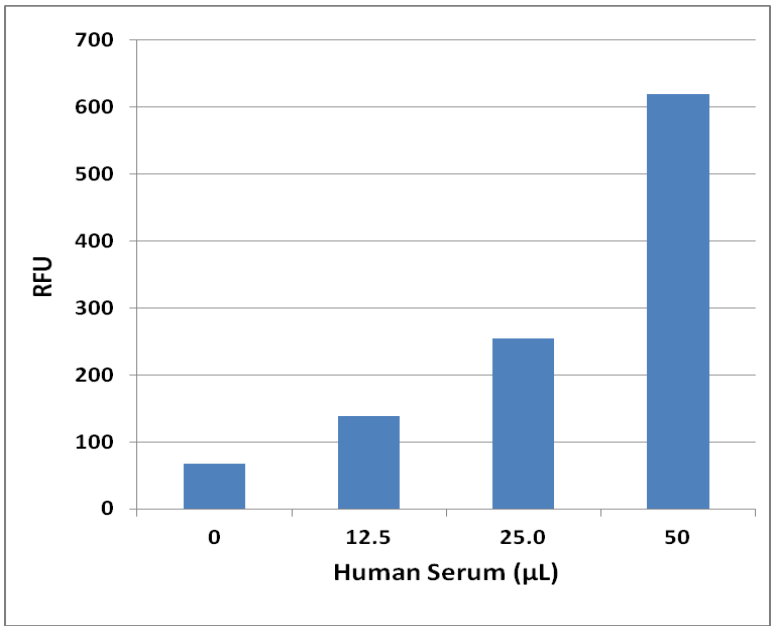


Figure 2: Beta Hexosaminidase Detection in Serum. Human serum was tested according to the Assay Protocol.

References

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3. Mark BL, Mahuran DJ, Cherney MM, Zhao D, Knapp S, James MN (2003). *J. Mol. Biol*. **327**: 1093–109.

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Recent Product Citations

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4. Jung, H. et al. (2022). Melittin-derived peptides exhibit variations in cytotoxicity and antioxidant, anti-inflammatory and allergenic activities. *Anim Cells Syst (Seoul)*. doi: 10.1080/19768354.2022.2099971.
5. Jung, H. et al. (2022). Mangifera Indica leaf extracts promote hair growth via activation of Wnt signaling pathway in human dermal papilla cells. *Anim Cells Syst*. doi: 10.1080/19768354.2022.2085790.
6. Lee, H.S. et al. (2021). Detoxification of Bee Venom Increases Its Anti-inflammatory Activity and Decreases Its Cytotoxicity and Allergenic Activity. *Appl Biochem Biotechnol*. doi: 10.1007/s12010-021-03653-2.

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