Recombinant Human Hexosaminidase
A/HEXA
Catalog Number: 6237-GH

**DESCRIPTION**

**Source**  Spodoptera frugiperda, Sf 21 (baculovirus)-derived
Met1-Thr529, with a C-terminal 6-His tag
Accession # P06865

**N-terminal Sequence Analysis**  Leu23

**Predicted Molecular Mass**  59 kDa

**SPECIFICATIONS**

**SDS-PAGE**  57-61 kDa, reducing conditions

**Activity**  Measured by its ability to hydrolyze 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide (4-MU-GlcNAc)
The specific activity is >1,250 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 under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 μg per lane.

**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: 100 mM Sodium Citrate, 250 mM NaCl, pH 4.5
- Recombinant Human Hexosaminidase A/HEXA (rhHEXA) (Catalog # 6237-GH)
- Substrate: 4-Methylumbelliferyl-N-Acetyl-β-D-glucosaminide (4-MU-GlcNAc) (Sigma, Catalog # M2133), 50 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 rhHEXA to 5 ng/μL in Assay Buffer.
2. Dilute Substrate to 800 μM in Assay Buffer.
3. Load into a plate 50 μL of 5 ng/μL rhHEXA, and start the reaction by adding 50 μL of 800 μM Substrate. For Substrate Blanks, load 50 μL of Assay Buffer and 50 μL of 800 μM Substrate.
4. Read plate at excitation and emission wavelengths of 365 nm and 445 nm, respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:

   \[
   \text{Specific Activity (pmol/min/μg)} = \frac{\text{Adjusted } V_{\max} \times (RFU/\min) \times \text{Conversion Factor}}{\text{amount of enzyme (μg)}}
   \]

   *Adjusted for Substrate Blank
   **Derived using calibration standard 4-Methylumbelliferone (4-MU) (Sigma, Catalog # M1381).

**Final Assay Conditions Per Well:**
- rhHEXA: 0.250 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**

β-hexosaminidases are enzymes involved in the hydrolysis of terminal N-acetyl-D-hexosamine residues in GM2 gangliosides and globo-sphingolipids in lysosomes (1-4). The enzymes are composed of two α and/or β subunits, which are coded by HEXA and HEXB genes, respectively. Different association of the α and β subunits gives rise to β-hexosaminidase isoforms A, B and S (Hex A, B and S) (5), which have the composition of αβ, ββ and αα, respectively. Our recombinant HEXA is presumably isoform Hex S, because only α subunit was expressed. Hex S is suggested to releases non-reducing end N-acetylgalactosamine residues from dermatan sulfate, chondroitin sulfate and sulfated glycolipid SM2 (6). Recombinant HEXA is also highly active on 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide (6). Mutations in HEXA and HEXB genes cause lysosomal lipid storage disorders. Specifically, mutations of HEXA cause Tay-Sachs disease, manifested by the harmful accumulation of ganglioside GM2 in tissues and nerve cells in the brain (7-10). Children with this disease usually die by age 4.

**References**