

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 $\alpha\beta$, $\beta\beta$ and $\alpha\alpha$, 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:

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Description

Source	<i>Spodoptera frugiperda</i> , Sf21 (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 pmoles/min/ μ g, as measured under the described conditions. See Activity Assay Protocol.
Endotoxin Level	<1.0 EU per 1 μ g of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 μ g per lane.
Formulation	Supplied as a 0.2 μ m filtered solution in Tris and NaCl. See Certificate of Analysis for details.

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. <ul style="list-style-type: none"> 6 months from date of receipt, -20 to -70 °C as supplied. 3 months, -20 to -70 °C under sterile conditions after opening.

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Activity Assay Protocol

Materials

- Assay Buffer: 100 mM Sodium Citrate, 250 mM NaCl, pH 4.5
- Recombinant human HEXA (R&D Systems, 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

- Dilute rhHEXA to 5 ng/ μ L in Assay Buffer.
- Dilute Substrate to 800 μ M in Assay Buffer.
- Load into a plate 50 μ L of 5 ng/ μ L rhHEXA, and start the reaction by adding 50 μ L of 800 μ M Substrate. For Substrate Blanks, load 50 μ L of Assay Buffer and 50 μ L of 800 μ M Substrate.
- Read plate at excitation and emission wavelengths of 365 nm and 445 nm, respectively, in kinetic mode for 5 minutes.
- Calculate specific activity:

$$\text{Specific Activity (pmoles/min}/\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* \text{ (RFU/min)} \times \text{Conversion Factor}^{**} \text{ (pmole/RFU)}}{\text{amount of enzyme } (\mu\text{g})}$$

*Adjusted for Substrate Blank

**Derived using calibration standard 4-Methylumbelliferone (4-MU) (Sigma, Catalog # 69580)

Final Assay Conditions Per Well

- rhHEXA: 0.250 μ g
- Substrate: 400 μ M

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NOT FOR USE IN HUMANS