Recombinant Mouse α-L-Iduronidase/IDUA

Catalog Number: 9348-GH

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Glu17-Ser634, with a C-terminal 6-His tag Accession # P48441
N-terminal Sequence Analysis	Glu17
Predicted Molecular Mass	70 kDa

SPECIFICATIONS

SDS-PAGE	83-95 kDa, reducing conditions
Activity	Measured by its ability to cleave a fluorogenic substrate, 4-Methylumbelliferyl α-L-iduronide. The specific activity is >7,500 pmol/min/ug, 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 visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Sodium Acetate, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM NaOAc, 150 mM NaCl, 0.02% Brij-35 (w/v), pH 3.5
- Developing Buffer: 0.1 M Tris, pH 9.0
- Recombinant Mouse α-L-Iduronidase/IDUA (rmIDUA) (Catalog # 9348-GH)
- Substrate: 4-methylumberlliferyl-α-L-Iduronide (Glycosynth, Catalog # 44076), 20 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rmIDUA to 0.2 µg/mL in Assay Buffer. Minimize the number of dilution steps to obtain the best activity results.
2. Dilute Substrate to 200 µM in Assay Buffer.
3. Combine equal volumes of 0.2 µg/mL rmIDUA and 200 µM Substrate. Include a Substrate Blank containing Assay Buffer and Substrate.
4. Incubate for 10 minutes at room temperature.
5. Dilute mixtures to 0.005 µg/mL rmIDUA in Developing Buffer.
6. In a plate load 100 µL of diluted mixtures.
7. Read at excitation and emission wavelengths of 365 nm and 445 nm (top read), respectively in endpoint mode.
8. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Fluorescence* (RFU) x Conversion Factor** (pmol/RFU)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

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

Final Assay Conditions

Per Well:

- rmIDUA: 0.0005 µg
- Substrate: 5 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

α-L-Iduronidase encoded by the IDUA gene is an important enzyme required for the lysosomal degradation of glycosaminoglycans (GAGS). It hydrolyzes the non-reducing terminal α-L-iduronic acid residues in GAGS including dermatan sulfate and heparan sulfate. Mature mouse IDUA shares 80% aa identity with human IDUA. Mutations in IDUA that result in enzymatic deficiency lead to the autosomal recessive disease mucopolysaccharidosis type I (MPS I) (1). MPS I can be classified as three clinical subtypes; Hurler syndrome, Hurler-Scheie syndrome, and Scheie syndrome with decreasing severity, respectively. MPS I causes progressive cellular, tissue and organ damage, and several clinical studies using enzyme replacement therapy show positive results (2, 3). Recently, the IDUA gene has been linked to osteoporosis (4, 5).

References:

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2. Wraith, J.E. (2005) Expert Opin. Pharmacother. **6**:489.
3. Jameson, E. (2016) Cochrane Database Syst. Rev. **4**: CD009354.
4. Kodric, K. *et al.* (2016) Wien Klin Wochenschr. **128**:480.
5. Niu, T. *et al.* (2016) J. Bone Miner. Res. **31**:358.

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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 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449