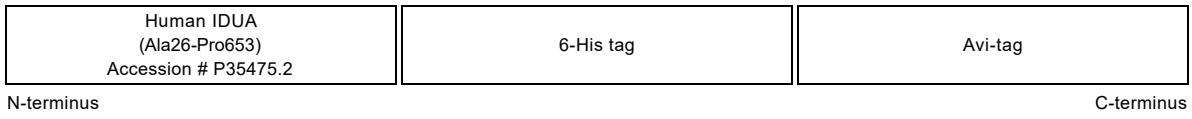
Biotinylated Recombinant Human α-L-Iduronidase/IDUA His-tag Avi-tag

Catalog Number: AVI11180

DESCRIPTION

Source

Chinese Hamster Ovary cell line, CHO-derived human alpha-L-Iduronidase/IDUA protein



N-terminal Sequence Analysis	Ala26
Predicted Molecular Mass	73 kDa

SPECIFICATIONS

SDS-PAGE	81-91 kDa, under reducing conditions
Activity	Measured by its binding ability in a functional ELISA. Biotinylated Recombinant Human α-L-Iduronidase/IDUA His-tag Avi-tag binds to Human alpha-L-Iduronidase/IDUA Antibody (Catalog # AF4119) with an ED_{50} of 0.800-8.00 ng/mL. Measured by its ability to cleave a fluorogenic substrate, 4-Methylumbelliferyl α-L-iduronide. The specific activity is >10,000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 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 Sodium Acetate, 150 mM NaCl, 0.02% Brij-35 (w/v), pH 3.5
- Development Buffer: 0.1 M Tris, pH 9.0
- Recombinant Human IDUA-Avi-tag (rhIDUA) (Catalog # AVI11180)
- Substrate: 4-methylumberlliferyl-α-L-Iduronide, 20 mM stock in DMSO
- Black 96-well Plate
- Plate Reader with Fluorescence Read Capability

Assay

1. Dilute rhIDUA to 0.2 µg/mL in Assay Buffer. Minimize the number of dilution steps to obtain the best activity results.
2. Dilute Substrate to 800 µM in Assay Buffer.
3. Combine equal volumes of 0.2 µg/mL rhIDUA and 800 µM Substrate. Include a Substrate Blank containing Assay Buffer and Substrate.
4. Incubate Reactions and Substrate Blank at room temperature for 10 minutes.
5. Dilute mixtures to 0.005 µg/mL in Development Buffer.
6. Load 100 µL of the diluted mixtures into a plate.
7. Read plate 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 from calibration standard 4-methylumbelliferone.

Final Assay Conditions

Per Well:

- rhIDUA: 0.0005 µg
- Substrate: 20 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.

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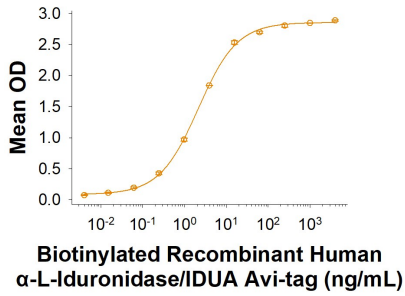
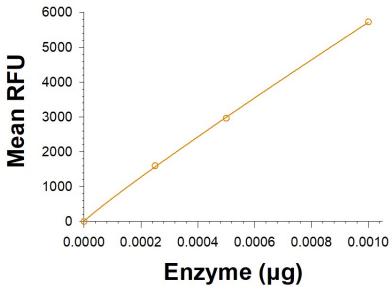
DATA

Binding Activity



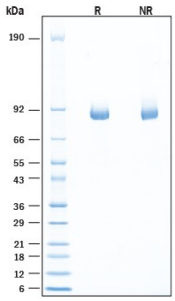
Biotinylated Recombinant Human α-L-Iduronidase/IDUA His-tag Avi-tag Protein Binding Activity. Measured by its binding ability in a functional ELISA. Biotinylated Recombinant Human α-L-Iduronidase/IDUA His-tag Avi-tag binds (Catalog # AVI11180) to Human alpha -L-Iduronidase/IDUA Antibody (Catalog # AF4119) with an ED_{50} of 0.800-8.00 ng/mL.

Enzyme Activity



Biotinylated Recombinant Human α-L-Iduronidase/IDUA His-tag Avi-tag Protein Enzyme Activity. Biotinylated Recombinant Human α-L-Iduronidase/IDUA His-tag Avi-tag Protein (Catalog # AVI11180) is measured by its ability to cleave a fluorogenic substrate, 4-Methylumbelliferyl α-L-iduronide.

SDS-PAGE



Biotinylated Recombinant Human α-L-Iduronidase/IDUA His-tag Avi-tag Protein SDS-PAGE. 2 µg/lane of Biotinylated Recombinant Human α-L-Iduronidase/IDUA His-tag Avi-tag Protein (Catalog # AVI11180) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 81-91 kDa, under reducing conditions.

BACKGROUND

a-L-Iduronidase is a member of the glycoside hydrolase family encoded by the IDUA gene (1). It is an important enzyme required for the lysosomal degradation of glycosaminoglycans (GAGS) and hydrolyzes the non-reducing terminal a-L-iduronic acid residues in GAGS including dermatan sulfate and heparan sulfate. Human IDUA is a 653 aa protein composed of a signal peptide removed in the lysosome for mature form and three domains: a triosephosphate isomerase barrel fold containing the catalytic site, a B-sandwich domain, and an Ig(Ig)-like domain. The protein has six reported N-glycosylation sites and the glycosylation status of the enzyme correlates with its catalytic activity (1). More than 55-disease associated missense mutations in the IDUA gene have been identified (1). Mutations in IDUA that result in enzymatic deficiency lead to the autosomal recessive disease mucopolysaccharidosis type I (MPS I) (2). 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 (3,4). More recently, the IDUA gene has been linked to osteoporosis (5, 6).

References:

1. Maita, N. *et al.* (2013) Proc. Natl. Acad. Sci. **110**:14628.
2. Scott, H.S. *et al.* (1995) Hum. Mutat. **6**:288.
3. Wraith, J.E. (2005) Expert Opin. Pharmacother. **6**:489.
4. Jameson, E. (2016) Cochrane Database Syst. Rev. **4**: CD009354.
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6. Niu, T. *et al.* (2016) J. Bone Miner. Res. **31**:358.

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