Recombinant Cynomolgus Monkey α-L-Iduronidase/IDUA His-tag

Catalog Number: 11281-GH

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived cynomolgus monkey alpha-L-Iduronidase/IDUA protein Ala26-Pro653 with a C-terminal 10-His tag Accession # XP_005554313.1
N-terminal Sequence Analysis	Ala26 & Glu27
Predicted Molecular Mass	72 kDa

SPECIFICATIONS

SDS-PAGE	81-90 kDa, under reducing conditions
Activity	Measured by its ability to cleave a fluorogenic substrate, 4-Methylumbelliferyl α-L-iduronide. The specific activity is >15,000 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 Cynomolgus alpha-L-Iduronidase/IDUA (rcynoIDUA) (Catalog # 11281-GH)
- Substrate: 4-methylumberlliferyl-α-L-Iduronide, 20 mM stock in DMSO
- Black 96-well Plate
- Fluorescent Plate Reader

Assay

1. Dilute rcynoIDUA 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 rcynoIDUA 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 reactions to 0.005 µg/mL rcynoIDUA in Developing Buffer. Dilute the Substrate Blank similarly.
6. Load 100 µL of the diluted reactions and Substrate Blank 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:

- rcynoIDUA: 0.0005 µg
- Substrate: 20 µM

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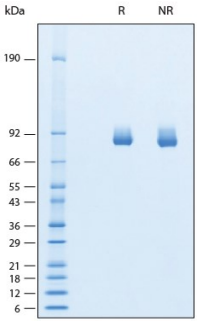
PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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.

DATA

SDS-PAGE



Recombinant Cynomolgus alpha-L-Iduronidase/IDUA Protein SDS-PAGE. 2 µg/lane of Recombinant Cynomolgus alpha-L-Iduronidase/IDUA Protein (Catalog # 11281-GH) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at ~81-90 kDa.

BACKGROUND

α-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 α-L-iduronic acid residues in GAGS including dermatan sulfate and heparan sulfate. Cynomolgus monkey 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 β-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 a hundred disease-associated variants in the IDUA gene have been identified (1, 2). Mutations in IDUA that result in enzymatic deficiency lead to the autosomal recessive disease mucopolysaccharidosis type I (MPS I) (3). MPS I can be classified into 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 (4, 5). More recently, the IDUA gene has been linked to osteoporosis (6, 7).

References:

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