**DESCRIPTION**

**Source**  
Mouse myeloma cell line, NS0-derived  
Ala26-Pro653 (Ala26Thr), with a C-terminal 10-His tag  
Accession # AAA81589

**N-terminal Sequence Analysis**  
Thr26

**Predicted Molecular Mass**  
71 kDa

**SPECIFICATIONS**

**SDS-PAGE**  
83 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/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 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 Human α-L-Iduronidase/IDUA (rhIDUA) (Catalog # 4119-GH)
- Substrate: 4-methylumbelliferyl-α-L-iduronide (Glycosynth, Catalog # 44076), 20 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 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 200 µM in Assay Buffer.
3. Combine equal volumes of 0.2 µg/mL rhIDUA 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 in Developing Buffer.
6. Load 100 µL of the diluted mixtures into a plate.
7. Read at excitation and emission wavelengths of 365 nm and 445 nm (top read), respectively in endpoint mode.
8. Calculate specific activity:

   Specific Activity (pmol/min/µg) = \[
   \frac{\text{Adjusted Fluorescence} \times \text{Conversion Factor}}{\text{Incubation time (min)} \times \text{amount of enzyme (µg)}}
   \]

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

**Final Assay Conditions**

- Per Well:
  - rhIDUA: 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. Mutations in IDUA that result in enzymatic deficiency lead to the autosomal recessive disease mucopolysaccharidosis type I (MPS I) (1). MPS I causes progressive cellular, tissue and organ damage, and several clinical studies using enzyme replacement therapy have shown promising benefits (2).

**References:**