

## DESCRIPTION

**Source** Mouse myeloma cell line, NS0-derived  
Met1-Thr651, with a C-terminal 6-His tag  
Accession # AAA52561

**N-terminal Sequence Analysis** Leu23

**Predicted Molecular Mass** 73 kDa

## SPECIFICATIONS

**SDS-PAGE** 75-85 kDa, reducing conditions

**Activity** Measured by its ability to hydrolyze 4-methylumbelliferyl-β-D-glucuronide.  
The specific activity is >4,000 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 Tris and NaCl. See Certificate of Analysis for details.

## Activity Assay Protocol

- Materials**
- Assay Buffer: 100 mM Sodium Acetate, pH 3.5
  - Recombinant Human β-Glucuronidase/GUSB (rhGUSB) (Catalog # 6144-GH)
  - Substrate: 4-Methylumbelliferyl β-D-glucuronide (Sigma, Catalog # M9130), 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**
1. Dilute rhGUSB to 4 ng/μL in Assay Buffer.
  2. Dilute Substrate to 2 mM in Assay Buffer.
  3. Load into a plate 50 μL of 4 ng/μL rhGUSB and start the reaction by adding 50 μL of 2 mM Substrate. For Substrate Blanks, load 50 μL of Assay Buffer and 50 μL of 2 mM Substrate.
  4. Read plate at excitation and emission wavelengths of 365 nm and 445 nm, respectively, in kinetic mode for 5 minutes.
  5. Calculate specific activity:

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

\*Adjusted for Substrate Blank

\*\*Derived using calibration standard 4-Methylumbelliferone (4-MU) (Sigma, Catalog # M1381).

- Final Assay Conditions**
- Per Well:
- rhGUSB: 0.200 μg
  - Substrate: 1 mM

## 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

Human β-Glucuronidase (EC 3.2.1.31) encoded by the GUSB gene is a lysosomal hydrolase involved in the stepwise degradation of glucuronic acid-containing glycosaminoglycans that include heparan sulfate, chondroitin sulfate and hyaluronan (1). The enzyme is only active on the glucuronic acid of the non-reducing end. The native protein has been reported as a tetrameric glycoprotein composed of identical subunits (1, 2). Mutations in the GUSB gene are linked to mucopolysaccharidosis type VII (3). Accumulation of partially degraded glycosaminoglycans, with glucuronic acid residues at the non-reducing termini, are usually found in the lysosomes of patients with the disease (3). It has also been reported that this enzyme may contribute to the depletion of chondroitin from cartilage and thereby facilitate the damage of joints in rheumatoid arthritis (4).

## References:

1. Shipley, J.M. *et al.* (1993) Am. J. Hum. Genet. **52**:517.
2. Oshima, A, *et al.* (1987) Proc. Natl. Acad. Sci. USA **84**:685.
3. Bell, C.E. Jr. *et al.* (1977) J. Clin. Invest. **59**:97.
4. Ortutay, Z. *et al.* (2003) Arthritis Rheum. **48**:2163.