

## Product Information

### **$\beta$ -Glucuronidase**

#### **Type IX-A**

from *Escherichia coli*

Product Number **G7396**

Storage Temperature  $-20\text{ }^{\circ}\text{C}$

CAS<sup>#</sup> 9001-45-0

EC 3.2.1.31

Synonyms:  $\beta$ -D-Glucuronide glucuronosohydrolase;  
GUS

#### **Product Description**

Glucuronidation, conjugation with glucuronic acid, by the human UDP-glucuronosyltransferase (UGT) family of enzymes plays an important role in the metabolic fate of many drugs and other xenobiotics. This biosynthetic reaction also has a role in the conjugation and excretion of endogenous substrates, such as steroids, bilirubin, and bile acids.<sup>1</sup> UGT activity results in the conjugation of glucuronic acid to substrates containing sulfhydryl, hydroxyl, aromatic amino, or carboxylic acid moieties. The glucuronides formed are more polar (water soluble) than the parent organic substrate and are generally excreted through the kidney.

$\beta$ -glucuronidase catalyzes the reaction:

$\beta$ -D-glucuronoside + H<sub>2</sub>O  $\leftrightarrow$  an alcohol + D-glucuronate

$\beta$ -Glucuronidase from *E. coli* is used for the enzymatic hydrolysis of  $\beta$ -glucuronides in urine and other fluids. It does not hydrolyze  $\alpha$ -glucuronides or  $\beta$ -glucosides.<sup>2</sup> The enzyme from *E. coli* has a high rate of hydrolytic activity and it retains this activity during hydrolysis better than similar enzymes that are more sensitive to changes in the concentration of  $\beta$ -glucuronide conjugates. The enzyme preparation from *E. coli* has been shown to be useful for determining the presence of androsterone, 17-hydroxycorticosteroids, and estriol in urine.<sup>3</sup> The optimal conditions for the enzymatic hydrolysis of  $\alpha$ -hydroxytriazolam, one of the major metabolites of triazolam in human urine, were determined using  $\beta$ -glucuronidase Type IX-A. It was found that a 90 minute incubation of 1 ml of urine with 100 units of the enzyme at 37  $^{\circ}\text{C}$  and pH 5.5-7.8, effectively hydrolyzed the  $\alpha$ -hydroxytriazolam given at the clinical dose.<sup>4</sup>

$\beta$ -Glucuronidase Type IX-A from *E. coli* is supplied as a powder lyophilized from 10 mM potassium phosphate, 1 mM ethylenediaminetetraacetic acid, and 1 mM dithiothreitol. Polyethylene glycol is added as a stabilizer.

Molecular Weight: ~290 kDa (tetramer)<sup>5</sup>  
68,259 Da (monomer)<sup>6</sup>

Optimal pH<sup>5</sup>: 6-7

Isoelectric point (pI)<sup>5</sup>: 4.8

Inhibitors:  
D-glucuronic acid  
(Product No. G5269)  
D-galacturonic acid  
D-glucaro-1, 4-lactone

#### Substrates:

5-Bromo-6-chloro-3-indolyl $\beta$ -D-glucuronide	B4532
6-Bromo-2-naphthyl $\beta$ -D-glucuronide	B6519
5-Bromo-4-chloro-3-indolyl $\beta$ -D-glucuronide sodium salt tablet	B8174
8-Hydroxyquinoline glucuronide	H1254
4-Methylumbelliferyl $\beta$ -D-glucuronide	M5664
4-Nitrophenyl $\beta$ -D-glucuronide	73677

#### Glucuronidase Activity:

1,000,000 - 5,000,000 units/gm protein

Unit Definition: One Sigma or modified Fishman unit will liberate 1.0  $\mu\text{g}$  of phenolphthalein from phenolphthalein glucuronide per hour at 37  $^{\circ}\text{C}$  at pH 6.8 (30 minute assay).

Unlike the enzyme preparation from snail (*Helix pomatia*) that naturally contains  $\beta$ -glucuronidase and sulfatase activities in almost equal amounts, the preparation of  $\beta$ -glucuronidase from *E. coli* is essentially free of sulfatase activity.

**Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

**Solubility**

When reconstituted to 5 mg/ml in 75 mM phosphate buffer, pH 6.8, a clear to slightly hazy solution results. Regardless of the cloudiness, the enzyme is active and should be useable for metabolite hydrolysis.

**Storage/Stability**

The product as supplied should be stored at  $-20\text{ }^{\circ}\text{C}$ .

A solution in 75 mM phosphate buffer, pH 6.8, ( $\geq 5\text{ mg/ml}$ ) may be stored at  $-20\text{ }^{\circ}\text{C}$  for up to 2 months with little or no loss of activity.

**References**

1. Tephly, T.R., *et al.*, *Adv. Pharmacol.*, **42**, 343-346 (1998).
2. *Methods of Enzymatic Analysis*, vol. 2, Hans Ulrich Bergmeyer, Ed., Academic Press, NY, 460-461, 929-943 (1974).
3. Graef, V., *et al.*, *Clin. Chem.*, **23**, 532-535 (1977).
4. Tsujikawa, K., *et al.*, *J. Health Sci.*, **50**, 286-289 (2004).
5. Kim, D-H, *et al.*, *Biol. Pharm. Bull.*, **18**, 1184-1188 (1995).
6. Jefferson, R.A., *et al.*, *Proc. Natl. Acad. Sci.*, **83**, 8447-8451 (1986).

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