

Product Information

β -Glucuronidase from limpets (*Patella vulgata*)

Aqueous solution, recombinant, expressed in proprietary host

SRE0093

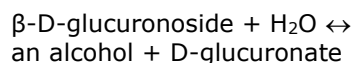
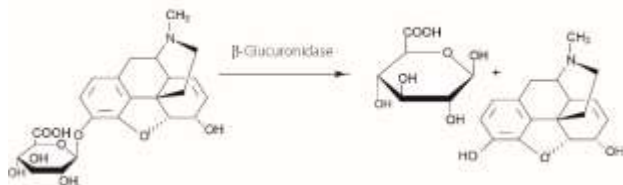
Product Description

CAS Registry Number: 9001-45-0

Enzyme Commission (EC) Number: 3.2.1.31

Synonyms: β -D-Glucuronide glucuronosohydrolase

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.¹ UGT activity results in the conjugation of glucuronic acid to substrates that contain sulfhydryl, hydroxyl, aromatic amino, or carboxylic acid moieties. The glucuronides formed are more polar (water-soluble) than the parent organic substrates, and are generally excreted through the kidney.

 β -glucuronidase catalyzes the general reaction:General β -Glucuronidase Hydrolysis Reaction β -Glucuronidase Hydrolysis Reaction for Morphine 3- β -glucuronide

β -Glucuronidases (GUS) are routinely used for the enzymatic hydrolysis of glucuronides from urine,^{2,3} plasma,^{4,5} and other fluids⁶ prior to analysis by enzyme immunoassay, mass spectrometry, HPLC, gas chromatography, or other methods. Typically, 1-20 units of glucuronidase are used per μL of plasma, urine, or bile for the enzymatic hydrolysis of glucuronides that are present in these samples.²⁻⁶ β -Glucuronidase from limpets has been shown to be a superior enzyme for the hydrolysis of the widest range of drug-glucuronides from urine.^{7,8}

Precautions and Disclaimer

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

Reagent

This recombinant β -glucuronidase, expressed in a proprietary host, is supplied as a solution in 100 mM potassium acetate, pH 5.2. It is free of detergents, carbohydrates, or any other components that may interfere with sample preparation and analysis. It is highly purified to maximize specific activity, and to eliminate monoacetylmorphine (MAM) esterase activity (6-monoacetylmorphine $\rightarrow$ morphine). Typical analysis at high enzyme concentration (50 units/ μL) exhibited <1% MAM esterase conversion after 4 hours at 60 °C.

Glucuronidase Activity: 100,000–200,000 units/mL

Unit Definition: One Sigma or modified Fishman unit will liberate 1.0 mg of phenolphthalein from phenolphthalein glucuronide per hour at 37 °C at pH 3.8 (30-minute assay).

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Storage/Stability

Store the product at 2-8 °C. When stored at 2-8 °C, the enzyme retains activity for at least two years. A representative sample of this product retained 92% activity after storage at 45 °C for 28 days.

Product Profile

Temperature and pH Activity Profiles

General temperature and pH activity profiles for Cat. No. SRE0093 are presented in Figures 1 and 2, respectively. Particular assays may require use with conditions different from the optima shown in these profiles. In both profiles, activity was measured as μg of phenolphthalein liberated per 10 minutes.

Figure 1. Temperature Profile of Cat. No. SRE0093 at pH 3.8

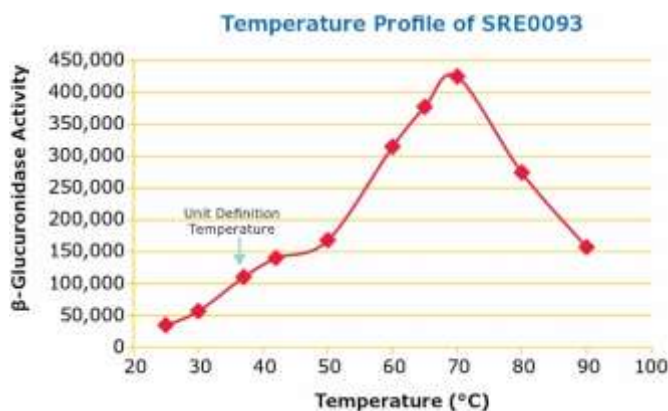
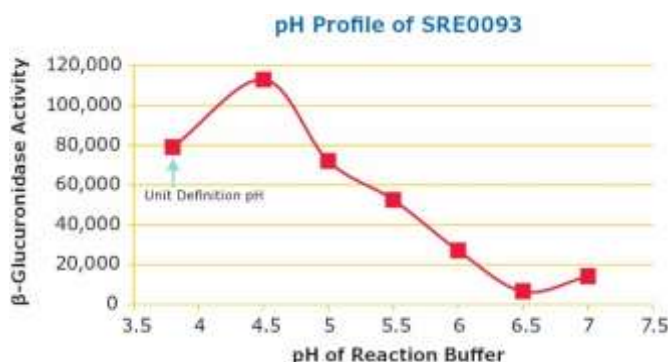


Figure 2. pH Profile of Cat. No. SRE0093 at 37 °C



Hydrolysis Data on Drug-Glucuronide Substrates

Figure 3 shows hydrolysis data of Cat. No. SRE0093 on different drug-glucuronide substrates. In particular, codeine 6- β -glucuronide is known as one of the most recalcitrant substrates in many drug analysis panels. Figure 4 shows the catalytic efficiency of the product in the hydrolysis of codeine 6- β -glucuronide to codeine. Hydrolysis data for additional drug classes are shown in Figures 5a-5e.

Figure 3. Hydrolysis of various drug-glucuronide substrates by Cat. No. SRE0093

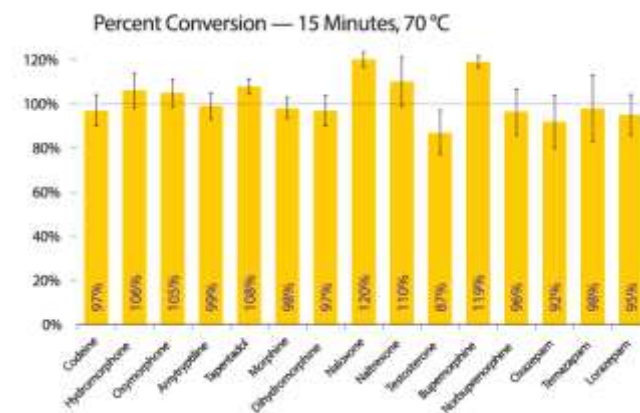
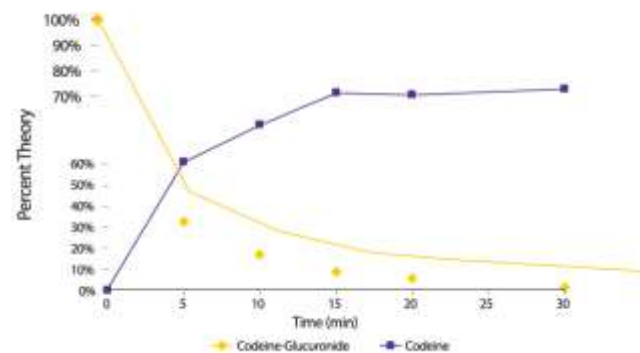


Figure 4. LC-MS Analysis of Enzymatic Hydrolysis of Codeine 6- β -Glucuronide with Cat. No. SRE0093



Conditions: 50 U/ μL enzyme in spiked urine at 1000 ng/mL glucuronide (nominal), 70 °C, pH 5.0.

Figures 5a-5e. Examples of enzymatic hydrolysis rates of various drug classes

Figure 5a. Opioid

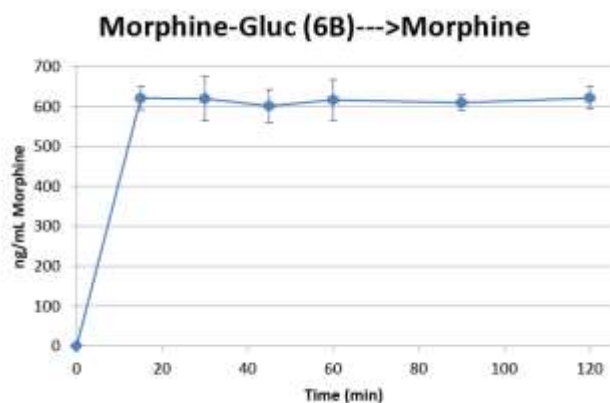


Figure 5b. Tricyclic Antidepressant

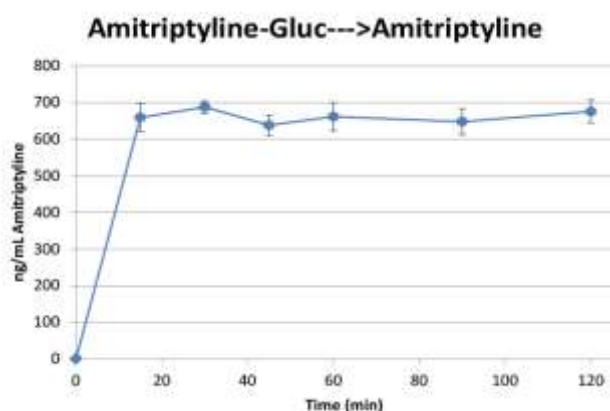


Figure 5c. Steroid

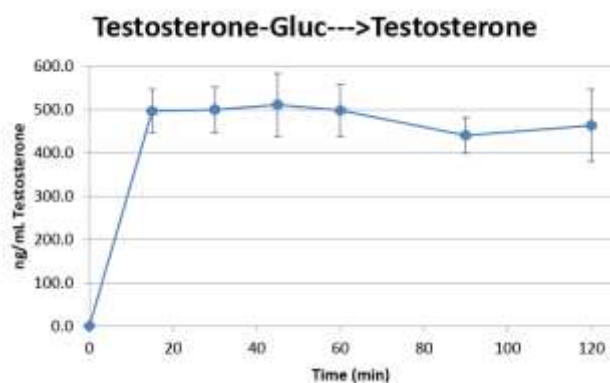


Figure 5d. Benzodiazepine

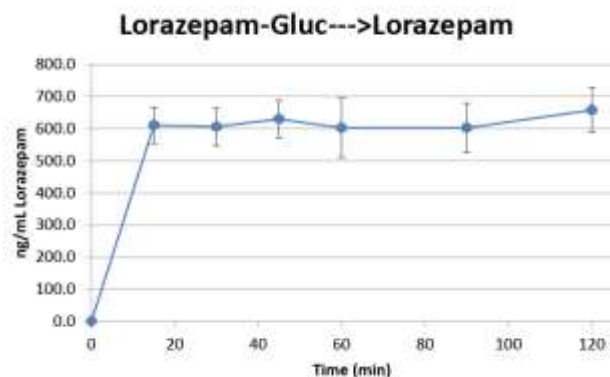
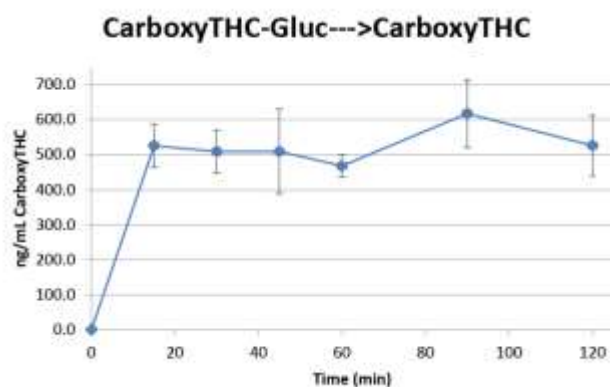


Figure 5e. Cannabinoid



Reaction conditions used (Figures 5a-5e):

- Glucuronide spike level at 1000 ng/mL (nominal) in synthetic urine
- Final concentration of Cat. No. SRE0093: 50 U/ μ L
- pH: 5.0
- Hydrolysis temperature: 70 $^{\circ}$ C
- Time points: 0, 15, 30, 45, 60, 90, and 120 minutes (reactions quenched with 20% TCA solution at 1:10 (v/v) TCA stock:sample)
- No sample preparation, injected "as is"

References

1. Tephly, T. R. *et al.*, *Adv. Pharmacol.*, **42**, 343-346 (1998).
2. Xu, X. *et al.*, *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, **780(2)**, 315-330 (2002).
3. Staimer, N. *et al.*, *Anal. Chim. Acta*, **444(1)**, 27-36 (2001).
4. Zhai, P. *et al.*, *Am. J. Physiol. Heart Circ. Physiol.*, **281(3)**, H1223-H1232 (2001).
5. Anderson, A. *et al.*, *Cancer Chemother. Pharmacol.*, **44(5)**, 422-426 (1999).
6. Nobilis, M. *et al.*, *J. Chromatogr. A*, **1031(1-2)**, 229-236 (2004).
7. Combie, J. *et al.*, *Clin. Chem.*, **28(1)**, 83-86 (1982).
8. Combie, J. *et al.*, *Res. Commun. Chem. Pathol. Pharmacol.*, **35(1)**, 27-41 (1982).

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