

## Product Information

### Fast $\beta$ -Glucuronidase, Recombinant

from limpets (*Patella vulgata*), expressed in proprietary host

Catalog Number **SRE0095**

Storage Temperature 2–8 °C

CAS RN 9001-45-0

EC 3.2.1.31

Synonym:  $\beta$ -D-Glucuronide glucuronosohydrolase

### 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 (GUS) enzymes are routinely used for the enzymatic hydrolysis of glucuronides from urine,<sup>2,3</sup> plasma,<sup>4,5</sup> and other fluids<sup>6</sup> prior to analysis by enzyme immunoassay, mass spectrometry, HPLC, gas chromatography, or other methods. Typically, between 1 and 20 units of glucuronidase is used per  $\mu$ L of plasma, urine, or bile, for the enzymatic hydrolysis of glucuronides present in these samples.<sup>2-6</sup>

$\beta$ -Glucuronidase from limpets has been shown to be a superior enzyme for the hydrolysis of drug-glucuronides from urine.<sup>7,8</sup>

This product 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.

This recombinant product is expressed in a proprietary host and highly purified to minimize protein content and eliminate monoacetylmorphine (MAM) esterase activity (6-monoacetylmorphine  $\rightarrow$  morphine). Typical analysis at high enzyme concentration (50 units/ $\mu$ L) exhibited <1% MAM esterase conversion after 4 hours at 60 °C.

This product is formulated with a higher enzyme concentration for applications that require ultra-fast hydrolysis rates.

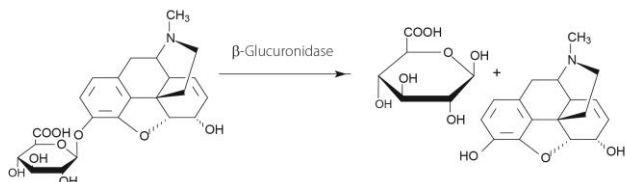
Glucuronidase Activity: 300,000–400,000 units/mL

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).

General  $\beta$ -Glucuronidase Hydrolysis Reaction

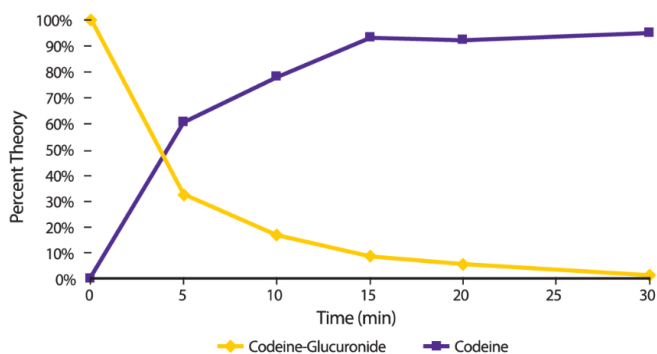


$\beta$ -Glucuronidase Hydrolysis Reaction for Morphine 3- $\beta$ -glucuronide



Codeine 6- $\beta$ -glucuronide is known as one of the most recalcitrant substrates in many drug analysis panels. The catalytic efficiency of this product was determined by monitoring the hydrolysis of codeine 6- $\beta$ -glucuronide to codeine (Figure 1).

**Figure 1.**  
LC-MS Analysis of Enzymatic Hydrolysis of Codeine 6- $\beta$ -Glucuronide with Catalog Number SRE0095.



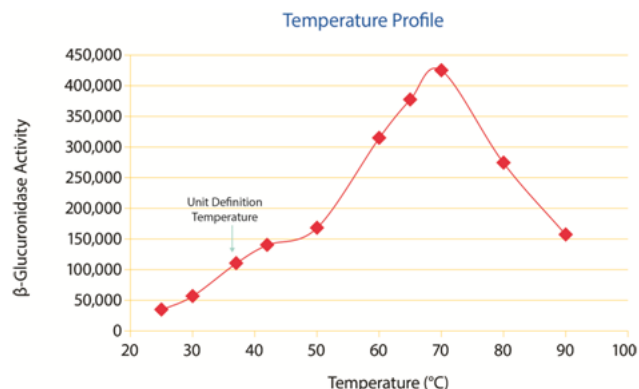
Complete hydrolysis by Catalog Number SRE0095 was observed in less than 30 minutes with 50 units/ $\mu$ L at 70 °C. Codeine-glucuronide spike level was at 1000 ng/mL in synthetic urine.

The suitability of SRE0095 was also demonstrated for hydrolysis of several other drug-glucuronide substrates:

- Morphine 6- $\beta$ -Glucuronide
- Hydromorphone Glucuronide
- Oxymorphone Glucuronide
- Lorazepam Glucuronide
- Oxazepam Glucuronide
- Testosterone Glucuronide.

Optimal temperature: 60–70 °C (see Figure 2)

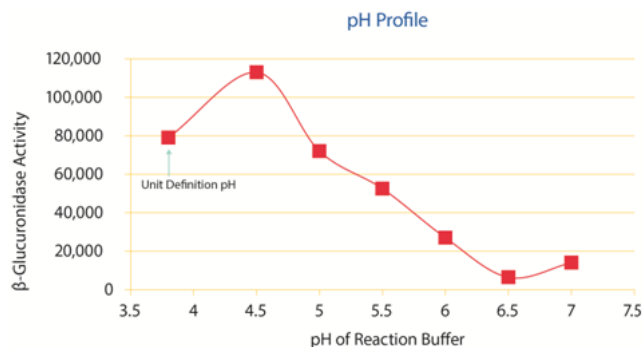
**Figure 2.**  
Temperature Profile of Catalog Number SRE0095 at pH 3.8.



Activity was measured as  $\mu$ g of phenolphthalein liberated per 10 minutes.

Optimal pH: 3.8–5.0 (see Figure 3)

**Figure 3.**  
pH Profile of Catalog Number SRE0095 at 37 °C.



Activity was measured as  $\mu$ g of phenolphthalein liberated per 10 minutes.

**Precautions and Disclaimer**

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.

**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.

## References

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DT, RBG, JMB, GCY, MAM 02/19-1