Product Information

Fast β-Glucuronidase, Recombinant

From limpets (*Patella vulgata*), expressed in proprietary host, aqueous solution

SRE0095

Product Description

CAS Registry Number: 9001-45-0

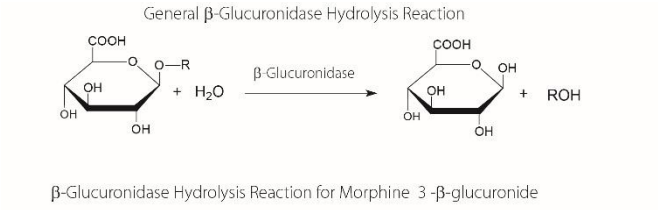
Enzyme Commission (EC) Number: 3.2.1.31

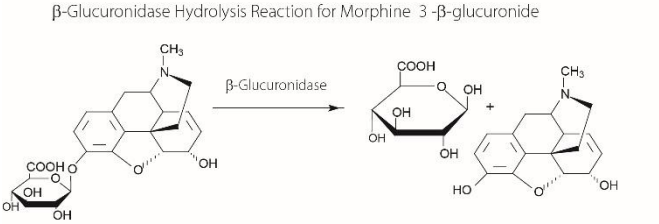
Synonyms: β-D-Glucuronide glucuronosohydrolase

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 containing 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:

β-D-glucuronoside + H_2O ↔
an alcohol + D-glucuronate





β-Glucuronidases (GUS) are routinely used for the enzymatic hydrolysis of glucuronides from urine,[2,3] plasma,[4,5] and other fluids[6] 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 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

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 also to eliminate monoacetylmorphine (MAM) esterase activity (6-monoacetylmorphine → morphine). Typical analysis at high enzyme concentration (50 units/µL) 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).

Storage/Stability

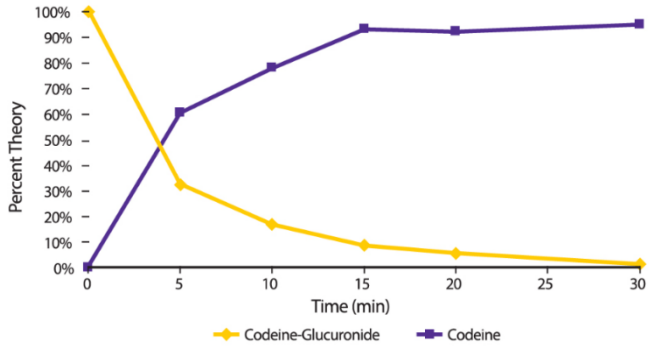
This product should be stored at 2-8 °C.

Product Profile

Hydrolysis of codeine 6-β-glucuronide

Codeine 6-β-glucuronide is known as one of the most recalcitrant substrates in many drug analysis panels. The catalytic efficiency of SRE0095 was determined by monitoring the hydrolysis of codeine 6-β-glucuronide to codeine (Figure 1).

Figure 1. LC-MS Analysis of Enzymatic Hydrolysis of Codeine 6-β-Glucuronide with Cat. No. SRE0095.



Complete hydrolysis by Cat. No. SRE0095 was observed in less than 30 minutes with 50 units/µL at 70 °C. Codeine-glucuronide spike level was at 1000 ng/mL in synthetic urine.

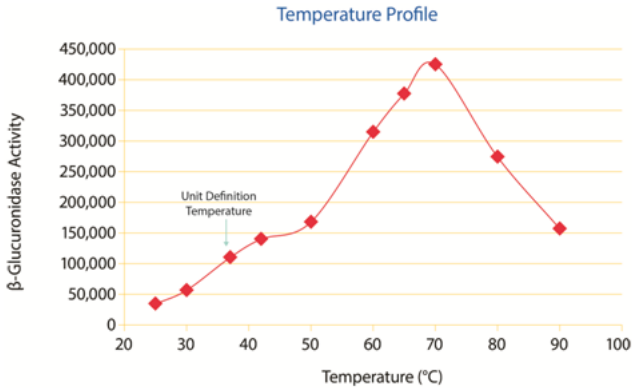
The suitability of SRE0095 was also demonstrated for hydrolysis of several other drug-glucuronide substrates:

- Morphine 6-β-Glucuronide
- Hydromorphone Glucuronide
- Oxymorphone Glucuronide
- Lorazepam Glucuronide
- Oxazepam Glucuronide
- Testosterone Glucuronide

Optimal temperature

Range: 60-70 °C (see Figure 2)

Figure 2. Temperature Profile of Cat. No. SRE0095 at pH 3.8.

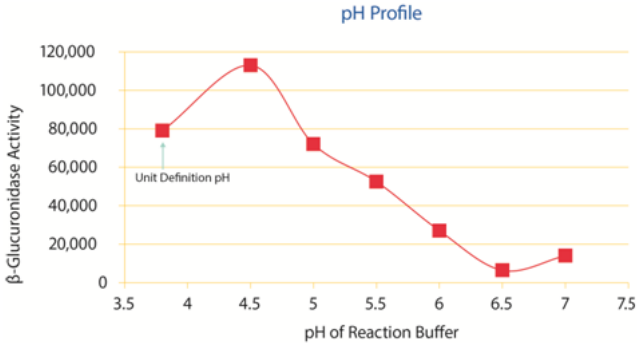


Activity was measured as µg of phenolphthalein liberated per 10 minutes.

Optimal pH

Range: 3.8-5.0 (see Figure 3)

Figure 3. pH Profile of Cat. No. SRE0095 at 37 °C.



Activity was measured as µg of phenolphthalein liberated per 10 minutes.

References

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SRE0095dat Rev 05/22 DT,RBG,JMB,GCY,MAM