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ProductInformation

UDP-Glucuronosyltransferase 1A8 Supersomes™ human, recombinant

Product Number **U 3008**
Storage Temperature –70 °C

EC 2.4.1.17
Synonyms: UGT1A8; UDP-glycosyltransferase

Product Description
This UDP-glucuronosyltransferase product is a microsomal fraction of insect cells (BTI-TN-5B1-4) infected with a baculovirus strain containing the cDNA for human UGT1A8.

Glucuronidation, conjugation with glucuronic acid, plays an important role in the metabolic fate of many drugs and other xenobiotics. Examining glucuronidation by the human UDP-glucuronosyltransferase (UGT) family of enzymes is essential when investigating the metabolism of new therapeutic agents or chemicals present in the environment. 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 substrate and are generally excreted through the kidney.

The UGT enzymes comprise a superfamily of integral membrane proteins of the endoplasmic reticulum that have been subdivided into two families, UGT1 and UGT2, based on the evolutionary divergence of their genes.[2] The enzymes of the UGT1A family play an important role in the metabolism of dietary constituents, phenols, and therapeutic drugs, and also the glucuronidation of bilirubin and iodothyronines. The enzymes of the UGT2B family are involved in the metabolism of bile acids, phenol derivatives, catechol-estrogens and steroids.[1,3] Although it is widely recognized that the liver is the major site of glucuronidation, it is now clear that UGT enzymes are also found in extra-hepatic tissues.

The product is supplied as a solution containing 100 mM Tris, pH 7.5.

UGT1A8 Specific Activity: minimum 0.3 unit/mg of protein (Lowry).

Unit Definition: One unit will transfer 1 nanomole of glucuronic acid from uridine-5'-diphosphoglucuronic acid to 7-hydroxy-4-trifluoromethylcoumarin per minute at pH 7.5 at 37 °C.

Storage/Stability
This UGT1A8 product ships on dry ice and should be stored at –70 °C. If not using the entire contents, aliquot to minimize freeze-thaw cycles. No loss of catalytic activity has been observed after the product has gone through 8 freeze/thaw cycles.

Procedure
7-Hydroxy-4-trifluoromethylcoumarin Glucuronidation Activity:
Reactions were performed in 0.2 ml volumes containing 0.25 mg/ml of UGT1A8 protein, 10 mM magnesium chloride, 0.025 mg/ml of alamethicin, 1 mM uridine diphosphoglucuronic acid, and 50 µM 7-hydroxy-4-trifluoromethylcoumarin in 50 mM Tris, pH 7.5. After incubation for 20 minutes at 37 °C, the reaction was stopped with 100 µl of 94% acetonitrile/6% acetic acid with subsequent centrifugation at 10,000 x *g*.

From the supernatant, 60 µl was injected on a 5 µm C18 HPLC column at 45 °C. There were three mobile phases: phase A (10% methanol), phase B (100% methanol), and phase C (30% acetonitrile and 1 mM perchloric acid). The initial gradiant was 80% phase A, 10% phase B, and 10% phase C. Over a 15 minute period, phase B was increased to 90%, while phase C remained constant at 10%. The reaction product, 7-hydroxy-4-trifluoromethylcoumarin glucuronide (Product Code T 6410) was detected by measuring absorbance at 325 nm and quantitated against a standard.

Notes: With respect to enzyme concentration, catalysis is linear up to at least 0.5 mg of UDP-glucuronosyl-transferase isozyme per ml. The glucuronidation of 7-hydroxy-4-trifluoromethylcoumarin is approximately linear for 30 minutes. Other substrates may not exhibit similar linearity.

References

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5. Bansal, S. K., and Gessner, T., A unified method for the assay of uridine diphosphoglucuronyl transferase activities toward various aglycones using uridine diphospho[U-^{14}C] glucuronic acid. Anal. Biochem., **109**, 321-329 (1980).
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