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ProductInformation

UDP-Glucuronosyltransferase 2B7 Isozyme
human, recombinant
expressed in baculovirus infected *Sf*9 cells

Catalog Number **U8504**
Storage Temperature –70 °C

E.C. 2.4.1.17
Synonyms: UGT2B7; UDP-glycosyltransferase

Product Description

This UDP-glucuronosyltransferase product is a microsomal fraction of *Sf*9 insect cells infected with a baculovirus strain containing the cDNA for human UGT2B7.

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. Human UGT2B7 is one of the most important UGT isozymes. It glucuronidates opioids, hyodeoxycholic acid, catechol-estrogens, androsterone, and many non-steroidal anti-inflammatory drugs (NSAIDs) with high efficiency.[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 in a solution containing 100 mM potassium phosphate, pH 7.4, with 20% (v/v) glycerol, 0.1 mM EDTA, and 1 mM DTT.

UGT2B7 Specific Activity: ≥0.067 unit/mg of protein

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

Storage/Stability

This UGT2B7 product ships on dry ice and the product is stored at –70 °C. The product, as supplied, is stable for at least 18 months. Substantial (>80%) activity has been observed after the product has gone through 5 freeze/thaw cycles.

Procedure

Two procedures have been reported for the measurement of UDP-glucuronosyltransferase activity. Both involve the use of ^{14}C-labeled uridine-5′-diphosphoglucuronic acid (^{14}C-UDPGA) as a substrate in the enzyme reaction and recovery of the ^{14}C-labeled glucuronide conjugate. The two methods differ in the method of isolation of the ^{14}C-labeled glucuronide reaction product. One method involves organic extraction[4] and the other uses thin-layer chromatography[5] (TLC) to separate the glucuronidated metabolite from ^{14}C-UDPGA and the other reaction components. The extraction method is much quicker to perform; however, when dealing with novel substrates, artifacts may be created by inefficient extraction of the glucuronidated product. An advantage to the TLC method is that the presence or absence of glucuronidated metabolites and ^{14}C-UDPGA can be visualized due to their different migration distances (R_f values).

In general, the optimal rate of glucuronidation will vary depending on the UGT enzyme, which is being tested. It is recommended that the investigator test the linearity of the assay with different protein and substrate concentrations.

Conditions routinely used for assay of the UGT2B7 enzyme:

Substrate: hyodeoxycholic acid
Final Substrate Concentration: 50 μM
Protein Concentration: 0.4 mg/ml
Time: 15 minutes

References

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2. Mackenzie, P.I. *et al*., The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. Pharmacogenetics, **7**, 255-269 (1997).
3. Burchell, B. *et al*., Specificity of human UDP-glucuronosyltransferases and xenobiotic glucuronidation. Life Sci., **57**, 1819-1831 (1995).
4. Matern, H. *et al*., Radioassay of UDP-glucuronosyltransferase activities toward endogenous substrates using labeled UDP-glucuronic acid and an organic solvent extraction procedure. Anal. Biochem., **219**, 182-188 (1994).
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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