Discovery Life Sciences
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Human UGT2B17 SUPERSOMES™

Catalog Number……..…456437
Lot Number.....……2406205

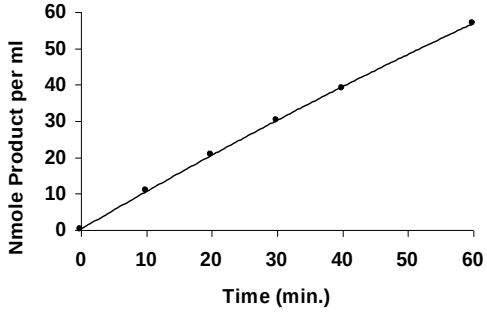
Storage Conditions..STORE AT -80°C
Date Released .....2024 July.
Expiration Date……..2034 July

Package Contents.....….....0.5 ml
Protein Content.....….....5.0 mg/ml in 0.1M Tris pH 7.5
Eugenol
Glucuronidation Activity.....……..…520 pmole/(min x mg protein)

This activity is catalyzed by UGT2B17, which is expressed from human UGT2B17 cDNA using a baculovirus expression system. Baculovirus infected insect cells (BTI-TN-5B1-4) were used to prepare these membranes. A membrane preparation using wild type virus (Catalog No. 456400) should be used as a control for native activities.

METHOD: A 0.2 ml reaction mixture containing 0.5 mg/ml protein, 1 mM uridine diphosphoglucuronic acid (UDPGA), 10 mM magnesium chloride, 0.025 mg/ml alamethicin and 200 μM Eugenol in 50 mM Tris (pH 7.5) was incubated at 37°C for 20 minutes. After incubation, the reaction was stopped by the addition of 50 ul 94% acetonitrile/6% glacial acetic acid and centrifuged (10,000 x g) for 3 minutes. 100 ul of the supernatant was injected into a 4.6 x 250 mm 5μ C18 HPLC column and separated at 45°C using a linear gradiant. The HPLC mobile phases consisted of 10% methanol (mobile phase A), 100% methanol (mobile phase B) and a solution consisting of 30% acetonitrile and 1 mM perchloric acid (mobile phase C). Initial HPLC conditions were 90% mobile phase A, and 10% of mobile phase C. The Eugenol glucuronide was eluted by increasing the concentration of Mobile phase B (100% methanol) to 70% over a 15 minute period, while keeping the concentration of mobile phase C constant at 10%. These conditions (20% mobile phase A, 70% mobile phase B and 10% mobile phase C) were held constant for an additional 3 minutes to elute the parent compound, prior to returning to initial conditions. The HPLC flow rate was 1ml per minute. The product was detected by its absorbance at 220 nm and quantitated by comparing to the absorbance of an external standard curve consisting of varying concentrations of Eugenol (parent compound). Note: The absorbance of the glucuronide metabolite was normalized to the absorbance of the standard curve by dividing the metabolite area by 0.8 (absorbance correction factor).

Time Course of Product Formation



ADVICE:

- Thaw rapidly in a 37°C water bath. Keep on ice until use.
- Aliquot to minimize freeze-thawing cycles. Less than 8% of the catalytic activity is lost after 10 freeze thaw cycles. Metabolite production with Eugenol is linear for at least 60 minutes (see graph above). Other substrates may not exhibit similar linearity with respect to incubation time.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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INSECT CELL MICROSOMES

HAZARD WARNING:

The product was produced using baculovirus (*Autographa californica*) infected insect cells (BTI-TN-5B1-4). This virus is not known to be pathogenic to humans or other mammals.

SAFETY INFORMATION:

Safety assessment indicates this product is not hazardous, therefore no SDS (Safety Data Sheet) is provided. Use standard laboratory practices for the handling and disposal of Biosafety Level 1 materials.

12 July 2024

Quality Assurance **Date**

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