Discovery Life Sciences
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Human UGT2B7 SUPERSOMES™

Catalog Number……...456427
Lot Number……2403316

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

Package Contents.....0.5 mL

Protein Content.....5.0 mg/mL in 0.1M Tris pH 7.5

7-Hydroxy-4-trifluoromethylcoumarin Glucuronidation Activity...970 pmole/(min x mg protein)

PRODUCT DESCRIPTION: This activity is catalyzed by UGT2B7 which is expressed from human UGT2B7 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.

ADVICE:

- Thaw rapidly in a 37°C water bath. Keep on ice until use.
- Aliquot to minimize freeze-thawing cycles. Less than 20% of the catalytic activity is lost after 5 freeze thaw cycles.
- Metabolite production using 7-hydroxy-4-trifluoromethylcoumarin glucoronidation as a substrate is linear with respect to enzyme concentration up to 0.5 mg/mL.
- Metabolite production with 7-hydroxy-4-trifluoromethylcoumarin is approximately linear for 15 minutes (see graph above). Other substrates may not exhibit similar linearity with respect to incubation time.
- Western immunoblotting indicates that the expressed UGT2B7 has similar mobility as UGT2B7 in human liver 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.

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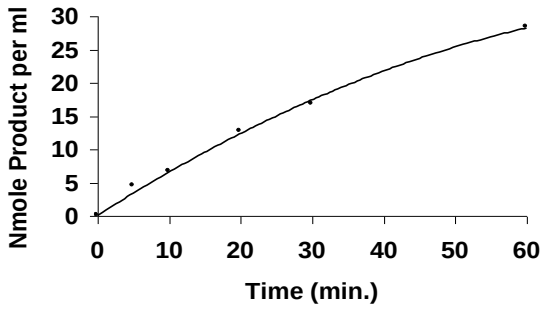
ASSAY METHOD: A 0.4 mL reaction mixture containing 0.25 mg/mL protein, 1 mM uridine diphosphoglucuronic acid (UDPGA), 10 mM magnesium chloride, 0.025 mg/mL alamethicin and 50 µM 7-hydroxy-4-trifluoromethylcoumarin in 50 mM tris (pH 7.5) was incubated at 37°C for 20 minutes. After incubation, the reaction was stopped by the addition of 100 µL 0.4 µM labetalol in acetonitrile with 0.1% formic acid and centrifuged (10,000 x g) for 3 minutes. The product was detected by its Q1 and Q3 mass with negative polarity and quantitated by comparing the peak area ratio to a standard curve of 4-trifluoromethyl-7-hydroxycoumarin glucuronide.

Time Course of Product Formation



ANALYTICAL METHOD:

Materials

Column	2.1 x 50 mm 5µm C18 HPLC
Mobile Phase A	0.1% Formic Acid in dH_2O
Mobile Phase B	0.1% Formic Acid in Acetonitrile

Mass Transitions of MRM

Compound	Q1 Mass (amu)	Q3 Mass (amu)
Analyte- 4-trifluoromethyl-7-hydroxycoumarin glucuronide	405.1 ±0.2	288.7 ±0.2
Internal Standard- Labetalol	327.3 ±0.2	176.0 ±0.2

Gradient Separation Conditions

Time (minute)	Flow Composition of Mobile Phase A (%)	Flow Composition of Mobile Phase B (%)	Flow Rate (µL /min)
0.0	95	5	750
2.5	5	95	750
2.6	95	5	750
3.6	95	5	750

20 May 2024

Quality Assurance **Date**

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