Discovery Life Sciences 6 Henshaw Street Woburn, MA 01801 Tel: (866) 838-2798 info@dls.com https://www.dls.com/

Human UGT2B7 SUPERSOMES™

Catalog Number......456427 **Lot Number**......2310031

Storage Conditions..STORE AT -80°C Date Released2023 October Expiration Date......2033 October

Package Contents......0.5 mL

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

7-Hydroxy-4-trifluoromethylcoumarin

Glucuronidation Activity......640 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.

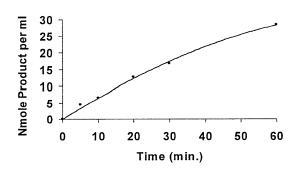
HAZARD WARNING: The product was produced using baculovirus (*Autographa californica*) infected insect cells (BTI-TN-5B1-4). This virus is not known to be pathogenic to humans or other mammals.

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 0.1% Formic Acid in dH ₂ O	
Mobile Phase A		
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

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

Quality Assurance

17 October 2023
Date