Discovery Life Sciences
6 Henshaw Street
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Human UGT1A3 SUPERSOMES™

Catalog Number……..456413
Lot Number.....……2407123

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

Package Contents.....…..…...0.5 mL
Protein Content.....……..…....5.0 mg/mL in 0.1M Tris pH 7.5
Estradiol 3-Glucuronidation Activity.…110 pmole/(min x mg protein)

PRODUCT DESCRIPTION: This activity is catalyzed by UGT1A3 which is expressed from human UGT1A3 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 10% of the catalytic activity is lost after 8 freeze thaw cycles.
- Metabolite production using estradiol as a substrate is linear with respect to enzyme concentration up to 1.5 mg/mL (highest concentration tested).
- Metabolite production with estradiol is linear for at least 40 minutes (see graph above). Other substrates may not exhibit similar linearity with respect to incubation time.

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.

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

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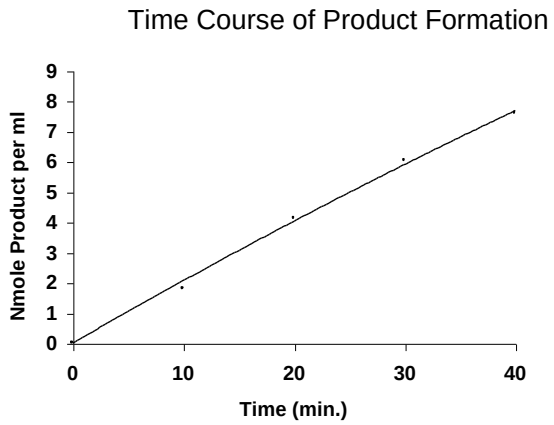
ASSAY METHOD: A 0.2 mL reaction mixture containing 1.0 mg/mL protein, 2 mM uridine diphosphoglucuronic acid (UDPGA), 10 mM magnesium chloride, 0.025 mg/mL alamethicin and 150 µM β-estradiol in 50 mM Tris (pH 7.5) was incubated at 37˚C for 30 minutes. After incubation, the reaction was stopped by the addition of 50 µL 1 µM estradiol- D3 glucuronide in acetonitrile with 0.1% formic acid and centrifuged (10,000 x g) for 3 minutes. The product was detected by LC-MS/MS using its Q1 and Q3 mass with negative polarity and quantitated by comparing the peak area ratio to a standard curve of estradiol 3-glucuronide.



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- Estradiol 3-Glucuronide	447.3 ±0.2	271.4 ±0.2
Internal Standard- Estradiol- D3 Glucuronide	450.3 ±0.2	274.3 ±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	10	90	800
0.2	10	90	800
0.4	25	75	800
2.0	35	65	800
2.2	98	2	800
2.5	98	2	800
2.7	10	90	800
2.8	10	90	800

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04 September 2024

Quality Assurance **Date**