Discovery Labware, Inc.
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CORNING

Human CYP4F3A + P450 Reductase + Cytochrome b_5 SUPERSOMES™

Catalog Number.....456273
Lot Number.....6271007

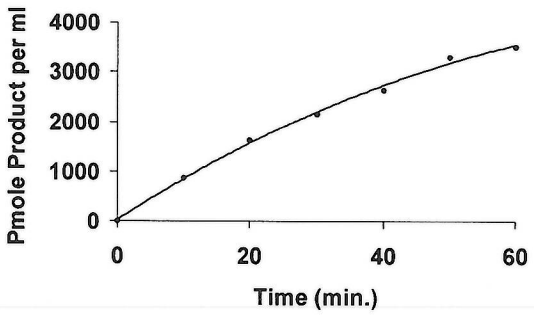
Storage Conditions..STORE AT -80°C
Date Released .....2016 November
Expiration Date.....2026 November

Package Contents.....0.25 nmole cytochrome P450 in 0.5 ml
Protein Content.....7.5 mg/ml in 100mM potassium phosphate (pH 7.4)
Cytochrome c Reductase Activity.....76 nmole/(min x mg protein)
Cytochrome b_5 Content.....430 pmol/mg
Cytochrome P450 Content.....500 pmol per ml
20-Hydroxy Leukotriene B_4 Activity.....87 pmol product/(min x pmol P450)

This activity is catalyzed by CYP4F3A which is expressed from human CYP4F3A cDNA using a baculovirus expression system. Baculovirus infected insect cells (BTI-TN-5B1-4) were used to prepare these microsomes. A microsome preparation using wild type virus (Catalog No. 456200 or 456201) should be used as a control for native activities.

METHOD: A 0.1 ml reaction mixture containing 1 pmole P450, 1.3 mM NADP+, 3.3 mM glucose-6-phosphate, 0.4 U/ml glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride and 30 uM Leukotriene B_4 in 50 mM phosphate buffer (pH 7.4) was incubated at 37°C for 5 min. After incubation, the reaction was stopped by the addition of 25 ul of 94% acetonitrile/6% glacial acetic acid and centrifuged (10,000 x g) for 3 minutes. 50 ul of the supernatant was injected into a 4.6 x 250 mm 5u C18 HPLC column and separated at 45°C initially with Mobile Phase A consisting of 30% acetonitrile with 1 mM perchloric acid in water changing to 70% methanol (Mobile Phase B) over 20 min. at a flow rate of 1.0 ml per min. The product was detected by its absorbance at 270 nm and compared to the absorbance of a standard curve for 20-hydroxy Leukotriene B_4.

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 20% of the catalytic activity is lost after 6 freeze thaw cycles.
- Metabolite production is linear with respect to enzyme concentration up to at least 8 pmol P450 per ml.
- Metabolite production with Leukotriene B_4 is approximately linear for 60 minutes (see graph above).

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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.

Quality Assurance

12 Sept, 2019
Date