

Discovery Life Sciences
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Human CYP4A11 + P450 Reductase SUPERSOMES™

Catalog Number.....456221
Lot Number.....2409260

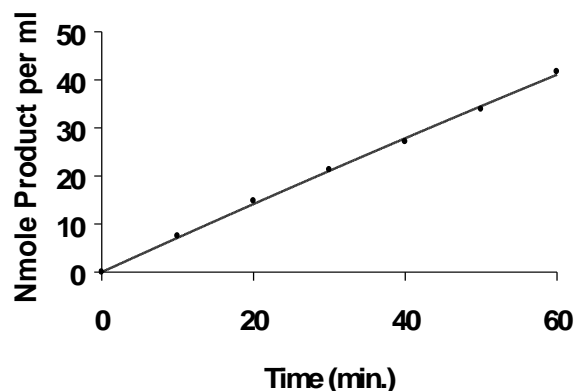
Storage Conditions..**STORE AT -80°C**
Date Released2024 October
Expiration Date.....2034 September

Package Contents.....0.5 nmole cytochrome P450 in 0.5 ml
Protein Content.....19 mg/ml in 100mM Tris (pH 7.5)
Cytochrome c Reductase Activity.....1000 nmole/(min x mg protein)
Cytochrome P450 Content.....1000 pmol per ml
Lauric Acid ω -Hydroxylase Activity..... 45 pmol product/(min x pmol P450)

This activity is catalyzed by CYP4A11 which is expressed from human CYP4A11 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. P201) should be used as a control for native activities.

METHOD: A 0.1 ml reaction mixture containing 2 pmoles 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 0.1 mM [¹⁴C]-lauric acid in 100mM Tris (pH 7.5) was incubated at 37°C for 20 min. After incubation, the reaction was stopped by the addition of 50 ul of 94% acetonitrile/6% glacial acetic acid and centrifuged (10,000 x g) for 3 minutes. 75 ul of the supernatant was injected into a 4.6 x 250 mm 5 μ C18 HPLC column and separated at 45°C with a mobile phase initially of 23% methanol, 23% acetonitrile with 1 mM perchloric acid in water changing to 100% methanol over 35 min. and at a flow rate of 1.0 ml per min. The retention times were approximately 17 min for the ω -hydroxylauric acid and 30 min for lauric acid. The product was detected by liquid scintillation counting.

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 with lauric acid is linear with respect to enzyme concentration up to at least 100 pmol P450 per ml.
- Metabolite production with lauric acid is approximately linear for 60 minutes (see graph above). Other substrates may not exhibit similar linearity with respect to incubation time.
- Western immunoblotting indicates the expressed CYP4A11 has similar mobility as CYP4A11 in human liver microsomes.
- Comparison of Western immunoblotting intensity and spectral P450 contents for this product and human lymphoblast-expressed CYP4A11 indicates that a substantial amount of apoprotein is found in this product.

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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.



01 October 2024

Quality Assurance

Date