

Discovery Life Sciences
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Human CYP2C8 + P450 Reductase + Cytochrome b₅ SUPERSOMES™

Catalog Number.....457230
Lot Number.....2911323
Date Released2024 July

LT Catalog Number.....PV6138
LT Lot Number.....2911323

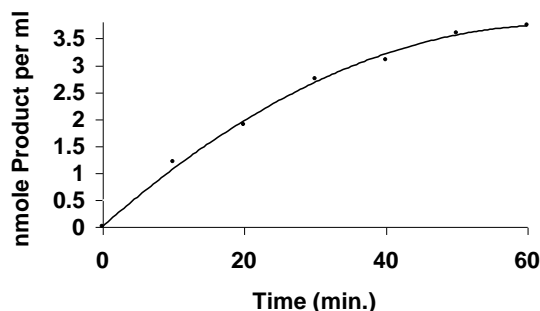
Storage Conditions.....STORE AT -80°C

Package Contents.....0.5 nmole cytochrome P450 in 0.5 ml
Protein Content.....10 mg/ml in 100mM potassium phosphate (pH 7.4)
Cytochrome c Reductase Activity.....170 nmole/(min x mg protein)
Cytochrome b₅ Content.....280 pmol/mg
Cytochrome P450 Content.....1000 pmol per ml
Paclitaxel 6 α -Hydroxylase Activity.....13 pmol product/(min x pmol P450)

This activity is catalyzed by CYP2C8 which is expressed from human CYP2C8 cDNA using a baculovirus expression system. Baculovirus infected insect cells (Hi5) were used to prepare these microsomes. These microsomes also contain cDNA-expressed human P450 reductase and human cytochrome b₅. A microsome preparation using wild type virus (Catalog No. 456201) should be used as a control for native activities.

METHOD: A 0.50 ml reaction mixture containing 10 pmole P450, 1.3mM NADP+, 3.3 mM glucose-6-phosphate, 0.4 U/ml glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride and 20 μ M paclitaxel (5 mM paclitaxel stock in ethanol) in 50 mM potassium phosphate (pH 7.4) was incubated at 37°C for 10 min. After incubation, the reaction was stopped by the addition of 150 μ l acetonitrile and centrifuged (10,000 x g) for 5 minutes. 100 μ l 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 60% methanol increasing to 70% methanol over 20 min and at a flow rate of 1.0 ml per min. The product was detected by its absorbance at 230 nm and quantitated by comparing the absorbance to a standard curve of 6 α -hydroxypaclitaxel. (Catalog No. B656).

Time Course of 6 α -Hydroxypaclitaxel 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 200 pmol P450 per ml.
- Metabolite production with paclitaxel is approximately linear for 20 minutes (see graph above). Other substrates may not exhibit similar linearity with respect to incubation time.

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INSECT CELL MICROSOMES SAFETY INFORMATION

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 RECOMMENDATIONS:

When using this product, follow good laboratory safety procedures:

Do not eat, drink or smoke.

Avoid contact with skin or eyes.

Do not inhale aerosols.

Do not pipette by mouth.

Wear suitable protective clothing, gloves and eye protection.

Steam sterilize product or treat product with a 10% solution of sodium hypochlorite prior to disposal.

Safety assessment indicates this product is hazardous; refer to SDS [Safety Data Sheet] for safety information.

Handle in accordance with good industrial hygiene and laboratory safety practices



17 July 2024

Quality Assurance

Date

Past periodic review date of 6/22/2024. Effective starting 4/4/2023. COA-457230 (version 2.1) COA Human CYP2C8 + P450
Reductase + Cytochrome b5 SUPERSOMES

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