Discovery Life Sciences
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Human CYP2C19 + P450 Reductase + Cytochrome b_5 SUPERSOMES™

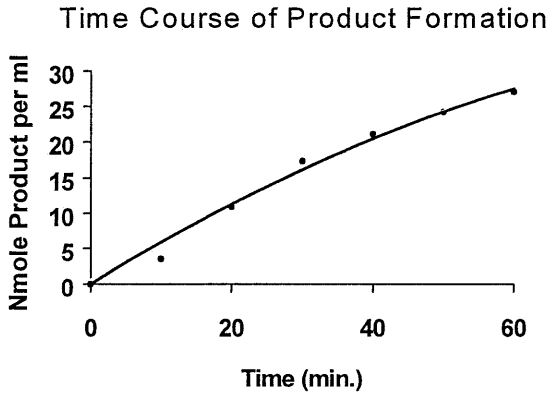
Catalog Number.....456259
Lot Number.....2311011

Storage Conditions...STORE AT -80°C
Date Released.....2023 December
Expiration Date.....2033 December

Package Contents.....0.5 nmole cytochrome P450 in 0.5 mL
Protein Content.....15 mg/mL in 100 mM potassium phosphate (pH 7.4)
Cytochrome c Reductase Activity.....200 nmole/(min x mg protein)
Cytochrome b_5 Content.....160 pmole/mg
Cytochrome P450 Content.....1000 pmole per mL
(S)-Mephenytoin 4'-Hydroxylase Activity..... 22 pmole product/(min x pmole P450)

This activity is catalyzed by CYP2C19 which is expressed from human CYP2C19 cDNA using a baculovirus expression system. Baculovirus infected insect cells (BTI-TN-5B1-4) were used to prepare these microsomes. These microsomes also contain cDNA-expressed human P450 reductase and human cytochrome b_5. A microsome preparation using wild type virus (Catalog No. 456201) should be used as a control for native activities.

METHOD: A 0.40 mL reaction mixture containing 16 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 0.1 mM (S)-Mephenytoin in 45 mM potassium phosphate (pH 7.4) was incubated at 37°C for 10 min. After incubation, the reaction was stopped by the addition of 100 µL 0.5 µM 4'-hydroxy-S-mephenytoin [D3] - in acetonitrile with 0.1% formic acid and centrifuged (14,000 x g) for 3 minutes. 5 µL of the supernatant was injected into a 2.1 x 50 mm 5 µm C18 HPLC column and separated at room temperature with a mobile phase initially increasing from 10% to 90% acetonitrile with 0.1% formic acid over 2.4 minutes, decreasing from 90% to 10% acetonitrile with 0.1% formic acid over 0.40 minutes, and then remaining at the initial conditions for 1.0 minutes. The flow rate was 0.40 mL per minute with positive polarity. The product, 4'-hydroxymephenytoin, was detected by its Q1 Mass of 235.1 ± 0.2 amu and Q3 Mass of 150.0 ± 0.2 amu and quantitated by comparing the atomic mass to a standard curve of 4'-hydroxymethenytoin.



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 pmole P450 per mL.
- Metabolite production with (S)-mephenytoin is approximately linear for 40 minutes (see graph above). Other substrates may not exhibit similar linearity with respect to incubation time.
- Expression of CYP2C19 is polymorphoric in human populations.
- CYP2C19 also has diclofenac 4'-hydroxylase activity.
- Western immunoblotting indicates the expressed CYP2C19 has the same mobility as CYP2C19 in human liver microsomes.
- Comparison of Western immunoblotting intensity and spectral P450 contents for this product and human lymphoblast-expressed CYP2C19 indicates that a substantial amount of apoprotein is found in this product.

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

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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 1% solution of sodium hypochlorite prior to disposal.

Quality Assurance

11 December 2023

Date