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Human CYP2C19 + P450 Reductase SUPERSOMES™

Catalog Number.....456219
Lot Number.....2403151

Storage Conditions..STORE AT -80°C
Date Released2024 April
Expiration Date.....2034 March

Package Contents.....0.5 nmole cytochrome P450 in 0.5 ml

Protein Content.....11 mg/mL in 100mM potassium phosphate (pH 7.4)

Cytochrome c Reductase Activity.....190 nmole/(min x mg protein)

Cytochrome P450 Content.....1000 pmol per mL

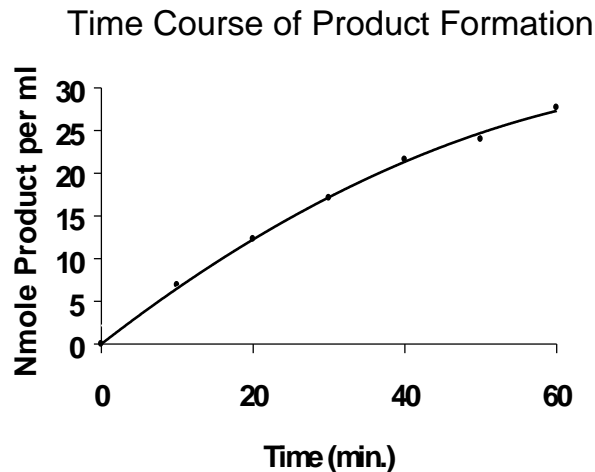
(S)-Mephenytoin 4'-Hydroxylase Activity...5.0 pmol product/(min x pmol P450)

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. 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 32 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 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 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% acetonitrile with 0.1% formic acid to 90% acetonitrile with 0.1% formic acid over 2.4 minutes then held at 10% acetonitrile with 0.1% formic acid for an additional 1.4 minutes with a positive polarity and at a flow rate of 0.40 mL per minute. 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'-hydroxymephenytoin.

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 (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 polymorphic 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

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.



22 April 2024

Quality Assurance

Date

Approved and current. Effective starting 12/22/2022. COA-456219 (version 2.1) COA Human CYP2C19 + P450 Reductase
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