Discovery Life Sciences
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Human CYP2E1 + P450 Reductase + Cytochrome b_5 SUPERSOMES™

Catalog Number.....456206
Lot Number.....2305309

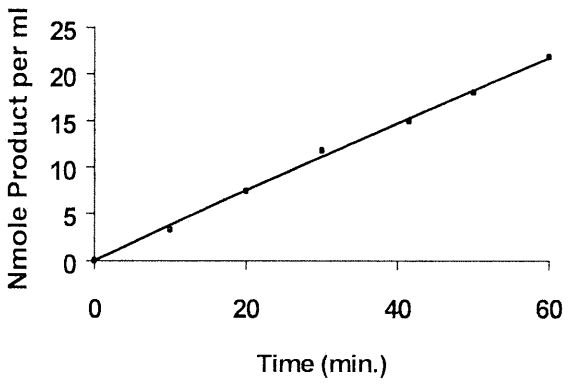
Storage Conditions..STORE AT -80°C
Date Released .....2023 July
Expiration Date.....2033 July

Package Contents.....1.0 nmole cytochrome P450 in 0.5 ml
Protein Content.....8.9 mg/ml in 100mM potassium phosphate (pH 7.4)
Cytochrome c Reductase Activity.....1700 nmole/(min x mg protein)
Cytochrome P450 Content.....2000 pmol per ml
Cytochrome b_5 Content.....220 pmol per mg protein
p-Nitrophenol Hydroxylase Activity.....27 pmol product/(min x pmol P450)

This activity is catalyzed by CYP2E1 which is expressed from human CYP2E1 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 0.5 ml reaction mixture containing 50 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 0.5 mM p-nitrophenol in 50 mM potassium phosphate (pH 7.4) was incubated at 37°C for 0.5 hr. A "blank" was performed with the same reaction mixture and adding microsomes immediately prior to stopping the reaction. After incubation, the reaction stopped by the addition of 0.1 ml 20% trichloroacetic acid and centrifuged (10,000 x g) for 1 minute. 0.5 ml of the supernatant was added to 0.25 ml 2 N NaOH and the absorbance measured at 535 nm (with water in the reference cuvette). The amount of product was determined by subtracting the absorbance of the "blank" from the absorbance of the microsome incubation and comparing to the absorbance of the product (p-nitrocatechol) under the same conditions.

Time Course of Product Formation



ADVICE

- **HUMAN CYP2E1 ACTIVITY IS HIGHLY INHIBITED BY LOW CONCENTRATIONS (0.001%) OF COMMON SOLVENTS SUCH AS DMSO, ETHANOL, ACETONE AND METHANOL.**
- Thaw rapidly in a 37°C water bath. Keep on ice until use
- Aliquot to minimize freeze-thawing cycles. Less than 10% of the catalytic activity is lost after 6 freeze thaw cycles.
- Metabolite production is linear with respect to enzyme concentration up to at least 160 pmol P450 per ml.
- Metabolite production with p-nitrophenol 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 CYP2E1 has the same mobility as CYP2E1 in human liver microsomes.

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- Comparison of Western immunoblotting intensity and spectral P450 contents for this product and human lymphoblast-expressed CYP2E1 indicates that a substantial amount of apoprotein is found in this product.

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

31 July, 2023
Date