

Human CYP2C8 + P450 Reductase SUPERSOMES™

Catalog Number.....456212
Lot Number.....4176001

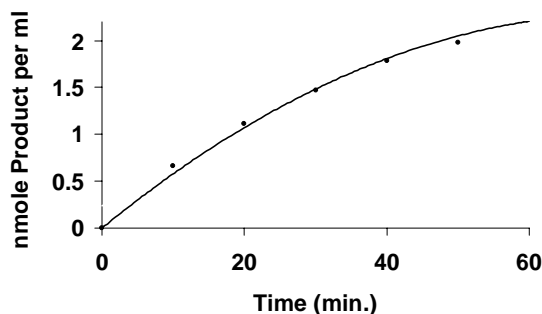
Storage Conditions..STORE AT -80°C
Date Released2014 July
Expiration Date.....2017 June

Package Contents.....1.0 nmole cytochrome P450 in 0.5 ml
Protein Content.....14 mg/ml in 100mM potassium phosphate (pH 7.4)
Cytochrome c Reductase Activity.....78 nmole/(min x mg protein)
Cytochrome P450 Content.....2000 pmol per ml
Paclitaxel 6 α -Hydroxylase Activity... ..18 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. 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 20 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 -hydroxy paclitaxel. (Catalog No. B656).

Time Course of
6 α -Hydroxy paclitaxel 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 7 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 1% solution of sodium hypochlorite prior to disposal.

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