

# Human CYP2C8 + P450 Reductase SUPERSOMES™

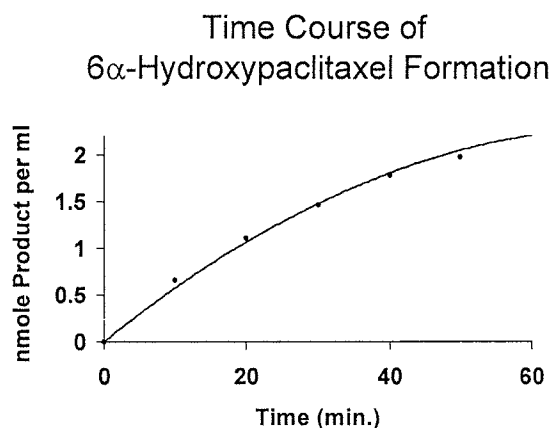
Catalog Number.....456212  
Lot Number.....6160002

Storage Conditions..STORE AT -80°C  
Date Released .....2016 June  
Expiration Date.....2019 June

Package Contents.....1.0 nmole cytochrome P450 in 0.5 ml  
Protein Content.....3.3 mg/ml in 100mM potassium phosphate (pH 7.4)  
Cytochrome c Reductase Activity.....250 nmole/(min x mg protein)  
Cytochrome P450 Content.....2000 pmol per ml  
Paclitaxel 6 $\alpha$ -Hydroxylase Activity... ..14 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  $\mu$ 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  $\mu$ l acetonitrile and centrifuged (10,000 x g) for 5 minutes. 100  $\mu$ l of the supernatant was injected into a 4.6 x 250 mm 5 $\mu$  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 $\alpha$ -hydroxypaclitaxel. (Catalog No. B656).



## 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.

**For research use only. Not for use in diagnostic or therapeutic procedures.**

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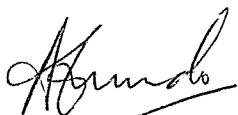
## INSECT CELL MICROSOMES SAFETY INFORMATION

### 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:

Handle in accordance with good industrial hygiene and laboratory safety practices.



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Quality Assurance

June 17, 2016

Date