

Human CYP1B1 + P450 Reductase SUPERSOMES™

Catalog Number.....456220
Lot Number.....5321002

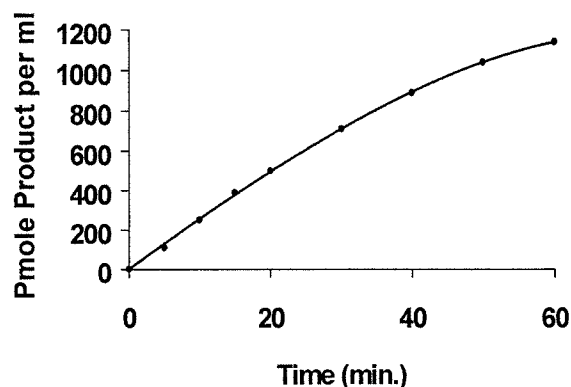
Storage Conditions..STORE AT -80°C
Date Released2015 November
Expiration Date.....2018 November

Package Contents.....0.5 nmole cytochrome P450 in 0.5 ml
Protein Content.....8.4 mg/ml in 100mM potassium phosphate (pH 7.4)
Cytochrome c Reductase Activity.....160 nmole/(min x mg protein)
Cytochrome P450 Content.....1000 pmol per ml
7-Ethoxyresorufin Deethylase Activity.....4.0 pmol product/(min x pmol P450)

This activity is catalyzed by CYP1B1 which is expressed from human CYP1B1 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 two ml reaction mixture containing 10 pmole P450, 1.3mM NADP+, 3.3mM glucose-6-phosphate, 0.4 U/ml glucose-6-phosphate dehydrogenase, 3.3mM magnesium chloride and 1 µg/ml 7-ethoxyresorufin in 100mM potassium phosphate (pH 7.4) was incubated at 37°C. The fluorescence of the product was determined continuously with excitation at 550 nm and emission at 586 nm in a spectrofluorometer. The increase in fluorescence in the first 0.5 minute (linear portion of the curve) was quantitated by comparing to the fluorescence of a resorufin standard.

Time Course of Product 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 6 freeze thaw cycles.
- Metabolite production is linear with respect to enzyme concentration up to at least 50 pmol P450 per ml.
- Metabolite production with 7-ethoxyresorufin is approximately linear for 40 minutes (see graph above). Other substrates may not exhibit similar linearity with respect to incubation time.
- Western immunoblotting indicates the expressed CYP1B1 is a single protein band with the predicted mobility of CYP1B1 based on its cDNA sequence.

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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.



Quality Assurance

November 25, 2015
Date