Discovery Labware, Inc.
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CORNING

Human CYP2A6 + P450 Reductase + Cytochrome b_5 SUPERSOMES™

Catalog Number……456254
Lot Number……4266008

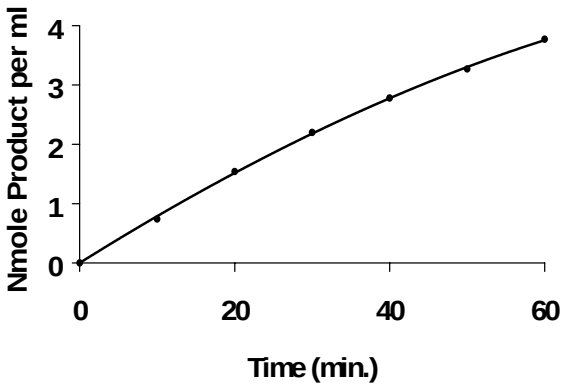
Storage Conditions..STORE AT -80°C
Date Released .....2014 October
Expiration Date……..2017 September

Package Contents……0.5 nmole cytochrome P450 in 0.5 mL
Protein Content……2.0 mg/mL in 100 mM Tris (pH 7.5)
Cytochrome c Reductase Activity……1200 nmole/(min x mg protein)
Cytochrome b_5 Content……570 pmole/mg
Cytochrome P450 Content……1000 pmole per mL
Coumarin 7-Hydroxylase Activity……16 pmole product/(min x pmole P450)

This activity is catalyzed by CYP2A6 which is expressed from human CYP2A6 cDNA using a baculovirus expression system. Baculovirus infected insect cells (BTI-TN-5B1-4) were used to prepare these microsomes. These microsomes also contain cDNA-expressed human P450 reductase and human cytochrome b_5. 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 10 pmole P450, 0.065 mM NADP+, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride and 0.4 mM coumarin in 50 mM Tris (pH 7.4) was incubated at 37°C for 10 min. After incubation, the reaction was stopped by the addition of 0.1 mL 20% trichloroacetic acid and centrifuged (10,000 x g) for 1 minute. 100 µL of the supernatant was added to 1.9 mL of 100 mM Tris (pH 9) and the fluorescence was determined with excitation at 368 nm and emission at 456 nm in a spectrofluorometer. The activity was quantitated by subtracting the fluorescence of the blank and comparing to a standard curve for umbelliferone (7-hydroxycoumarin).

Time Course of Product Formation



ADVICE

- **HUMAN CYP2A6 ACTIVITY IS INHIBITED BY PHOSPHATE.**
- 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 80 pmole P450 per mL.
- Metabolite production with coumarin is approximately linear for 30 minutes (see graph above). Other substrates may not exhibit similar linearity with respect to incubation time.
- Some lots of NADP+ and glucose-6-phosphate may support lower levels of CYP2A6 activity.
- Western immunoblotting indicates the expressed CYP2A6 has the same mobility as CYP2A6 in human liver microsomes.
- Comparison of Western immunoblotting intensity and spectral P450 contents for this product and human lymphoblast-expressed CYP2A6 indicates that a small 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
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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