Discovery Life Sciences
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Human CYP2B6 + P450 Reductase + Cytochrome b_5 SUPERSOMES™

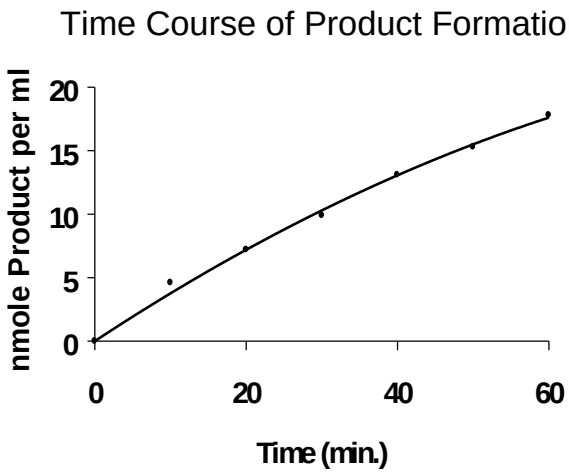
Catalog Number……..456255
Lot Number.....……2402169

Storage Conditions..STORE AT -80°C
Date Released .....2024 February
Expiration Date.……2034 February

Package Contents.....…...….....0.5 nmol cytochrome P450 in 0.5 mL
Protein Content.....…..……13 mg/mL in 100mM potassium phosphate (pH 7.4)
Cytochrome c Reductase Activity…....2060 nmol/(min x mg protein)
Cytochrome b5 Content...……..……180 pmol per mg
Cytochrome P450 Content...……..……..1000 pmol per mL
7-Ethoxy-4-Trifluoromethyl-coumarin Deethylase Activity.……17 pmol product/(min x pmol P450)

7-Ethoxy-4-trifluoromethylcoumarin deethylase activity has replaced 7-ethoxycoumarin deethylase activity for this enzyme. 7-Ethoxy-4-trifluoromethylcoumarin deethylase is more sensitive and specific for CYP2B6. We have found that specific activities can vary with different lots of substrate. This activity is catalyzed by CYP2B6 which is expressed from human CYP2B6 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 0.25 mL reaction mixture containing 10 pmol 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 0.1 mM 7-ethoxy-4-trifluoromethylcoumarin in 50 mM potassium phosphate (pH 7.4) was incubated at 37°C for 15 minutes. After incubation, the reaction was stopped by the addition of 50 µL 20% trichloroacetic acid and centrifuged (10,000 x g) for 1 minute. 100µl of the supernatant was added to 1.9 mL 100 mM Tris (pH 9) and the fluorescence was determined with excitation at 410 nm and emission at 510 nm in a spectrofluorometer. The activity was quantitated by subtracting the fluorescence of the blank and comparing to a standard curve for 7-hydroxy-4-trifluoromethylcoumarin.



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 with 7-ethoxy-4-trifluoromethylcoumarin is linear with respect to enzyme concentration up to at least 80 pmol P450 per ml.
- Metabolite production with 7-ethoxy-4-trifluoromethylcoumarin is approximately linear for 50 minutes (see graph above). Other substrates may not exhibit similar linearity with respect to incubation time.
- Western immunoblotting indicates the expressed CYP2B6 has the same mobility as CYP2B6 in human liver microsomes.
- Comparison of Western immunoblotting intensity and spectral P450 contents for this product and human lymphoblast-expressed CYP2B6 indicates that a substantial 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.

29 February 2024

Quality Assurance **Date**

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