

Human CYP19 + P450 Reductase SUPERSOMES™

Catalog Number.....456260
Lot Number.....8355001

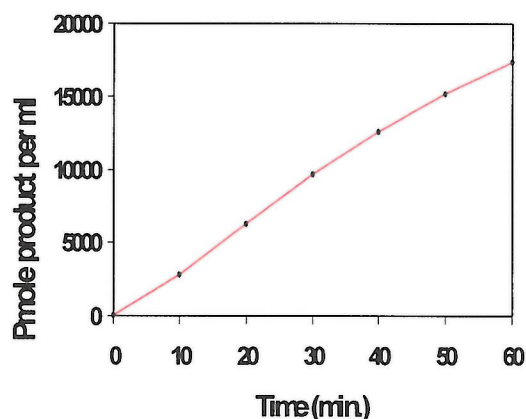
Storage Conditions..STORE AT -80°C
Date Released2019 January
Expiration Date.....2029 January

Package Contents.....0.5 nmole cytochrome P450 in 0.5 mL
Protein Content..... 8.2 mg/mL in 100 mM potassium phosphate (pH 7.4)
Cytochrome c Reductase Activity.....170 nmole/(min x mg protein)
Cytochrome P450 Content..... 1000 pmole per mL
Aromatase Activity.....7.2 pmole product/(min x pmole P450)

This activity is catalyzed by human CYP19 which is expressed from human CYP19 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. A microsome preparation using wild type virus (Catalog No. 456200 or 456201) should be used as a control for native activities.

METHOD: A 0.25 mL reaction mixture containing 25 pmole 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 0.05 mM testosterone in 100 mM potassium phosphate (pH 7.4) was incubated at 37°C for 20 min. After incubation, the reaction was stopped by the addition of 125 μ L acetonitrile and centrifuged (10,000 x g) for 3 minutes. 50 μ L of the supernatant was injected into a 4.6 x 250 mm 5 μ m C18 HPLC column and eluted isocratically at 45°C with a mobile phase of 60% water and 40% acetonitrile and at a flow rate of 1.5 mL per min. The product was detected by its absorbance at 200 nm and quantitated by comparing the absorbance to a standard curve of (beta)-estradiol.

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 pmole P450 per mL.
- Metabolite production with testosterone is approximately linear for 40 minutes (see graph above).

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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.


Quality Assurance


Date

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