Discovery Life Sciences
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# Human CYP2D6∗1 + P450 Reductase SUPERSOMES™

**Catalog Number**……..456217
**Lot Number**.....…….2403282

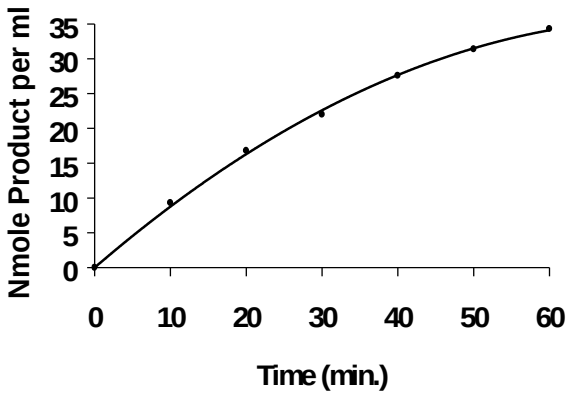
**Storage Conditions. STORE AT -80°C**
**Date Released** .....2024 April
**Expiration Date**……..2034 April

**Package Contents**.....…..…. 0.5 nmole cytochrome P450 in 0.5 mL
**Protein Content**.....…… 11 mg/mL in 100 mM potassium phosphate (pH 7.4)
**Cytochrome c Reductase Activity**…..... 3200 nmole/(min x mg protein)
**Cytochrome P450 Content**……..…. 1000 pmole per mL
**(+/-)-Bufuralol 1'-Hydroxylase Activity**… 70 pmole product/(min x pmole P450)

This activity is catalyzed by CYP2D6*1 which is expressed from human CYP2D6*1 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 1.0 mL reaction mixture containing 10 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 100 μM (+/-)-bufuralol in 100 mM potassium phosphate (pH 7.4) was incubated at 37°C for 10 minutes. After incubation, 100 uL of 70% perchloric acid was added and the mixture was centrifuged at 12000 x g to pellet the protein. A portion of the supernatant was injected into a 4.6 x 250 mm 5μm C18 HPLC column and separated at 45°C with a mobile phase of 30% acetonitrile, 1 mM perchloric acid at a flow rate of 1.5 mL per minute. The retention time of the product was approximately 4 minutes. The fluorescence of the product was measured in the flow cell of a spectrofluorometer with excitation at 252 nm and emission at 302 nm. The response was quantitated by comparing to a standard curve of product (1'-hydroxybufuralol).

Time Course of Product Formation




## ADVICE

- Human CYP2D6 also catalyzes the hydroxylation of debrisoquine and a variety of commonly used pharmaceuticals such as dextromethorphan. The expression of CYP2D6 in human populations is polymorphic.
- This preparation contains CYP2D6 protein levels and CYP2D6 catalytic activity substantially higher than that present in human liver microsomes.
- Thaw rapidly in a 37°C water bath. Keep on ice until use.
- Aliquot to minimize freeze-thawing cycles. Less than 10% of the catalytic activity is lost after 7 freeze thaw cycles.
- Metabolite production is linear with respect to enzyme concentration up to at least 100 pmole P450 per mL.
- Metabolite production with (+/-)-bufuralol is approximately linear for 30 minutes (see graph above). Other substrates may not exhibit similar linearity with respect to incubation time.
- Western immunoblotting indicates the expressed CYP2D6 has the same mobility as CYP2D6 in human liver microsomes.
- Comparison of Western immunoblotting intensity and spectral P450 contents for this product and human lymphoblast-expressed CYP2D6 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

### 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.

24 April 2024

**Quality Assurance** **Date**