Discovery Life Sciences
6 Henshaw Street
Woburn, MA 01801
Tel: (866) 838-2798
info@dls.com
https://www.dls.com/

# Human CYP2C9*1 ($Arg_{144}$) + P450 Reductase + Cytochrome $b_5$ SUPERSOMES™

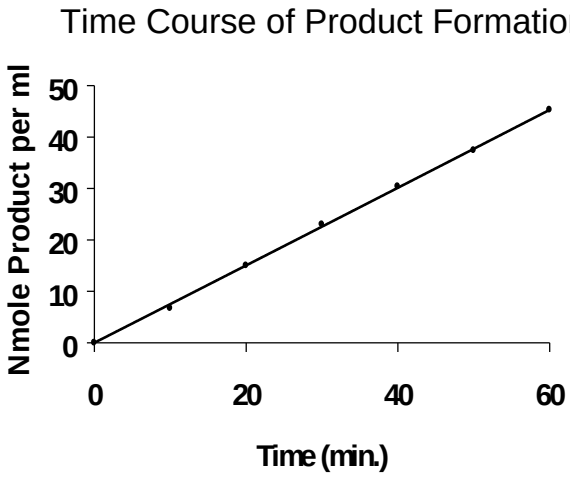
**Catalog Number**……..456258
**Lot Number**.....…….2401060

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

**Package Contents**.....……..……0.5 nmole cytochrome P450 in 0.5 ml
**Protein Content**.....……..……..3.2 mg/ml in 100mM Tris (pH 7.5)
**Cytochrome c Reductase Activity**……...800 nmole/(min x mg protein)
**Cytochrome $b_5$ Content**.....…… 500 pmol/mg
**Cytochrome P450 Content**...……....1000 pmol per ml
**Diclofenac 4'-Hydroxylase Activity**...……..130 pmol product/(min x pmol P450)

This activity is catalyzed by CYP2C9*1 ($Arg_{144}$) which is expressed from human CYP2C9*1 ($Arg_{144}$) 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 0.50 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 0.2 mM diclofenac in 100mM Tris (pH 7.5) was incubated at 37°C for 10 min. After incubation, the reaction was stopped by the addition of 250 µl of 0.5µM 4'Hydroxydiclofenac-13C6 in 0.1% Formic Acid in Acetonitrile and centrifuged (10,000 x g) for 3 minutes. 5 µl of the supernatant was injected into a 2.1 x 50 mm 5µm C18 HPLC column and separated at room temperature with a mobile phase initially increasing from 30% acetonitrile with 0.1% formic acid to 95% acetonitrile with 0.1% formic acid over 2 minutes then held at 30% acetonitrile with 0.1% formic acid for additional 1.5 minutes with a negative polarity and at a flow rate of 0.4 mL per minute. The product, 4-Hydroxydiclofenac, was detected by its Q1 Mass of 312.0±0.2 amu and Q3 Mass of 268.0±0.2 amu and quantitated by comparing the atomic mass to a standard curve of 4-Hydroxydiclofenac




## ADVICE

- Thaw rapidly in a 37°C water bath. Keep on ice until use.
- CYP2C9 is less active in phosphate buffers.
- 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 200 pmol P450 per ml.
- Metabolite production with diclofenac is approximately linear for at least 60 minutes (see graph above). Other substrates may not exhibit similar linearity with respect to incubation time.
- Western immunoblotting indicates the expressed CYP2C9 has the same mobility as CYP2C9 in human liver microsomes. Comparison of Western immunoblotting intensity and spectral P450 contents for this product and human lymphoblast-expressed CYP2C9 indicates that a substantial amount of apoprotein is found in this product.

***For Research Use Only. Not for use in diagnostic or therapeutic procedures.***

Discovery Life Sciences
6 Henshaw Street
Woburn, MA 01801
Tel: (866) 838-2798
info@dls.com
https://www.dls.com/

# 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.

28 February 2024

**Quality Assurance** **Date**

***For Research Use Only. Not for use in diagnostic or therapeutic procedures.***