

## Product Information

### CYTOCHROME P450 CYP2C9\*3 ISOZYME Human, Recombinant Microsomes with Cytochrome P450 Reductase

Product Number **C 4360**  
Storage Temperature  $-70\text{ }^{\circ}\text{C}$

#### Product Description

The microsomal product is prepared from insect cells (BTI-TN-5B1-4) infected with recombinant baculovirus containing cDNA inserts for the human cytochrome P450 isozyme and human cytochrome P450 reductase.

Cytochrome P450 enzymes are a superfamily of heme containing monooxygenases, which are found primarily in the mammalian liver and catalyze the oxidative metabolism of xenobiotics. This metabolism is the initial step in the biotransformation and elimination of a wide variety of drugs and environmental pollutants from the body. These reactions are achieved through a mixed monooxygenase system with the general EC number of 1.14.14.1.<sup>1</sup> CYP2C9\*3 (Leu 359) is an allelic variant of CYP2C9. The frequency of this allele is 6%, 0.5%, and 2.6% for Caucasian-American, African-American, and Chinese-Taiwanese populations, respectively.

The cytochrome P450 enzymes range in molecular weight between 45 to 60 kDa. The human CYP2C9 isozyme is less active in phosphate buffers.

The product is supplied as 1.0 nmole of cytochrome P450 isozyme in 0.5 ml of 100 mM Tris-HCl buffer, pH 7.5. A substantial amount of apoprotein is detected. Protein content, cytochrome c reductase activity, and diclofenac 4'-hydroxylase activity of the microsomes are reported on a lot-to-lot basis.

#### Precautions and Disclaimer

This product is for laboratory research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

#### Preparation Instructions

1. Quickly thaw at  $37\text{ }^{\circ}\text{C}$  using a water bath. Keep on ice until ready to use.
2. If not using entire contents, aliquot to minimize freeze-thaw cycles. Generally, 80% or more of the catalytic activity is retained after 6 freeze-thaw cycles.
3. Store aliquots at  $-70\text{ }^{\circ}\text{C}$ .

#### Storage/Stability

The product is shipped on dry ice and should be stored at  $-70\text{ }^{\circ}\text{C}$ . The product as supplied is stable for at least 24 months. For prolonged storage, freeze in working aliquots at  $-70\text{ }^{\circ}\text{C}$ . Avoid repeated freezing and thawing.

#### Procedure

##### Diclofenac 4'-Hydroxylase Activity:

A 0.5 ml reaction containing the following was incubated at  $37\text{ }^{\circ}\text{C}$  for 10 minutes:

- 20 pmoles cytochrome P450 isozyme
- 1.3 mM NADP<sup>+</sup>
- 3.3 mM glucose-6-phosphate
- 0.4 U/ml glucose-6-phosphate dehydrogenase
- 3.3 mM magnesium chloride
- 0.2 mM diclofenac
- 100 mM Tris-HCl buffer, pH 7.5

The reaction was stopped with 100  $\mu\text{l}$  of a 94% acetonitrile/6% glacial acetic acid and centrifuged (10,000 x g) for 3 minutes. A 100  $\mu\text{l}$  aliquot of the supernatant was injected into a 4.6 x 250 mm 5  $\mu\text{m}$  C18 HPLC column and separated at  $45\text{ }^{\circ}\text{C}$ . A 20 minute gradient from 0-100% Phase B was used at a flow rate of 1.0 ml per minute. Mobile Phase A: 30% methanol, 20% acetonitrile with 1 mM perchloric acid in water and Mobile Phase B: 100% methanol. The product was detected by measuring absorbance at 280 nm and comparison was made to a standard curve of 4'-hydroxydiclofenac (Product No. H 3661).

Notes: With respect to enzyme concentration, catalysis is linear up to at least 200 pmoles of cytochrome P450 isozyme per ml. Hydroxylation of diclofenac is approximately linear for 60 minutes. Other substrates may not exhibit similar linearity. NADPH may be substituted for the NADPH generating system, which consists of NADP<sup>+</sup>, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase.

## References

1. Enzyme Nomenclature, IUBMB, Academic Press (1992).
2. Anzenbacher, P., and Anzenbacherova, E., Cytochromes P450 and metabolism of xenobiotics. *Cell Mol. Life Sci.*, **58**, 737-47 (2001).
3. Guengrich, F.P. Cytochrome P450: Structure, Mechanism and Biochemistry (2<sup>nd</sup> Edition), Chapter 14. Ortiz de Montellano, P.R. (ed.) Plenum Press, (New York, NY: 1995).
4. Shimamoto, J., et al., Lack of differences in diclofenac (a substrate for CYP2C9) pharmacokinetics in healthy volunteers with respect to the single CYP2C9\*3 allele, *Eur. J. Clin. Pharmacol.*, **56**, 65-8 (2000).

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