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Product Information

Cytochrome P450 CYP2C9 isozyme human, recombinant microsomes with Cytochrome P450 Reductase expressed in baculovirus infected *Sf*9 cells

Catalog Number **C5107** Storage Temperature –70 °C

Product Description

The microsomal product is prepared from insect cells (*Sf*9) infected with recombinant baculovirus containing cDNA inserts for the human cytochrome P450 isozyme and rabbit cytochrome P450 reductase. Metabolism by endogenous insect cytochromes P450 has not been detected.

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 1.14.14.1.¹

The CYP2C9 isoform is homologous to endotheliumderived hyperpolarizing factor (EDHF) of porcine coronary arteries, which generates reactive oxygen species and modulates vascular tone. Weakly acidic lipophilic substrates are preferred, while sulfaphenazole is a known inhibitor.

The cytochrome P450 enzymes range in molecular mass between 45–60 kDa.

The product is supplied as 1.0 nmole of cytochrome P450 isozyme in a solution of 100 mM Tris, pH 7.5. Cytochrome c reductase activity, turnover activity, and protein content of the microsomes are reported on a lot-to-lot basis.

Cytochrome P450 Content: 500 pmole of cytochrome P450 (spectral analysis) per milligram protein.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

- 1. Quickly thaw at 37 °C using a water bath. Keep on ice until ready to use.
- 2. If not using entire contents, aliquot to minimize freeze-thaw cycles.
- 3. Store aliquots at –70 °C.

Storage/Stability

The product is shipped on dry ice and should be stored at -70 °C. The product, as supplied, remains active for at least 18 months. For prolonged storage, freeze in working aliquots at -70 °C. Avoid repeated freezing and thawing.

Procedure

In general, $\leq 1\%$ of the total reaction volume may be organic solvent. Any solvent at a concentration between 1–5% will have a serious effect on P450 activity. If it is necessary to use concentrations >1%, acetonitrile should be used since it has less of an effect on substrate metabolism. DMSO should never be used, since a concentration as low as 0.2% may inhibit certain types of cytochrome P450 activity.

References

- 1. Enzyme Nomenclature, IUBMB, Academic Press (1992).
- Anzenbacher, P., and Anzenbacherova, E., Cytochromes P450 and metabolism of xenobiotics. Cell Mol. Life Sci., 58, 737-747 (2001)
- Lewis, D.F.V., On the recognition of mammalian microsomal cytochrome P450 substrates and their characteristics. Biochem. Pharmacol., 60, 293-306 (2000).
- Giancarlo, G.M. et al., Relative contributions of CYP2C9 and 2C19 to phenytoin 4-hydroxylation *in vitro*: inhibition by sulfaphenazole, omeprazole, and ticlopidine. Eur. J. Clin. Pharmacol., **57**, 31-36 (2001).
- Redman, A.R., Implications of cytochrome P450 2C9 polymorphism on warfarin metabolism and dosing. Pharmacotherapy, **21**, 235-242 (2001).

- Fleming, I. et al., Endothelium-derived hyperpolarizing factor synthase (Cytochrome P450 2C9) is a functionally significant source of reactive oxygen species in coronary arteries. Circ. Res., 88, 44-51 (2001).
- Leeman, T. et al. Cytochrome P450TB (CYP2C): a major monooxygenase catalyzing diclofenac 4'-hydroxylation in human liver. Life Science, 52, 9-34 (1992).
- Guengrich, F.P., Cytochrome P450: Structure, Mechanism and Biochemistry (2nd Edition), Ortiz de Montellano, P.R. (ed.) Plenum Press (New York, NY: 1995) Chapter 14.

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