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Product Information

CYTOCHROME P450 CYP2C9 ISOZYME Human, Recombinant

Product Code **C 7817**
Storage Temperature $-70\text{ }^{\circ}\text{C}$

Product Description

A human, recombinant protein produced from over-expressed plasmid in *E. coli*. This cytochrome P450 isozyme has been modified at the N-terminal to allow expression in *E. coli*, but these changes do not cause any significant differences in substrate specificity.

The microsomal cytochrome P450 enzymes, found primarily in the endoplasmic reticulum of liver tissue, 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 advantages of using a purified cytochrome P450 enzyme include the lack of interfering activities present in microsomal or tissue samples, and the flexibility to optimize component ratios of cytochrome P450, NADPH-P450 reductase, and cytochrome b_5 for specific applications.

The cytochrome P450 enzymes range in molecular weight between 45 to 60 kDa.

Vial content: 100 μg protein (≥ 1 nanomole of P450) in a solution containing 20 mM potassium phosphate buffer, pH 7.4, 0.2 mM EDTA, 1 mM DTT and 20% (v/v) glycerol.

Cytochrome P450 content: Minimum 10 nanomole of P450 (spectral analysis)/mg protein (BCA)

Purity: $\geq 50\%$ by SDS-PAGE

Dichlofenac hydroxylation activity: Minimum 250 units/mg protein (BCA)

Unit Definition: One unit will convert 1.0 nanomole of dichlofenac to hydroxydichlofenac per minute at pH 7.4 at $37\text{ }^{\circ}\text{C}$.

Precautions

In general, $\leq 1\%$ of the total reaction volume may be organic solvent. Any solvent at a concentration between 1 and 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.

Storage/Stability

The cytochrome P450 product is stored at $-70\text{ }^{\circ}\text{C}$. The product as supplied is stable for at least 18 months.

Procedure

Approximately 1-2 μg should be loaded per lane for immunoblotting.

Reconstitution methods^{2,3,4} and specific substrate assays⁵ for the CYP2C9 isozyme have been published.

References

1. Enzyme Nomenclature, IUBMB, Academic Press (1992).
2. Brian, W.R., et al. Biochemistry, **29**, 11280-11292 (1990).
3. Gillam, E.M., et al. Arch. Biochem. Biophys., **317**, 374-384 (1995).
4. Ingelman-Sundberg, M., et al. Biochem. Biophys. Res. Comm., **221**, 318-322 (1996).
5. Leeman, T., et al. Life Science, **52**, 29-34 (1992).

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