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ProductInformation

CYTOCHROME P450 CYP4A11 ISOZYME
Human, Recombinant
Microsomes with Cytochrome P450 Reductase

Product Number **C4610**
Storage Temperature –70 °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. The recombinant CYP4A11 has the same mobility (western immunoblotting) as CYP4A11 in human liver microsomes.

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

The product is supplied as 0.5 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 lauric acid ω-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 °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 °C.

Storage/Stability

The product is shipped on dry ice and should be stored at –70 °C. The product as supplied is stable for at least 24 months. For prolonged storage, freeze in working aliquots at –70 °C. Avoid repeated freezing and thawing.

Procedure

Lauric Acid ω-Hydroxylase Activity:
A 0.1 ml reaction containing the following was incubated at 37 °C for 20 minutes:
2 pmoles cytochrome P450 isozyme
1.3 mM $NADP^+$
3.3 mM glucose-6-phosphate
0.4 U/ml glucose-6-phosphate dehydrogenase
3.3 mM magnesium chloride
0.1 mM ^{14}C-lauric acid
100 mM Tris-HCl, pH 7.5
The reaction was stopped with 50 µl of 94% Acetonitrile/6% glacial acetic acid and centrifuged (10,000 x *g*) for 3 minutes. A 100 µl aliquot of the supernatant was injected into a 4.6 x 250 mm 5 µm C18 HPLC column and separated at 45 °C. A 35 minute gradient from 0-100% Phase B was used at a flow rate of 1.0 ml per minute. Mobile Phase A: 23% methanol, 23% acetonitrile with 1 mM perchloric acid in water and Mobile Phase B: 100% methanol. The retention time for the hydroxylated product was approximately 17 minutes compared to 30 minutes for the lauric acid. Both were detected by liquid scintillation counting.

Notes: With respect to enzyme concentration, catalysis is linear up to at least 100 pmoles of cytochrome P450 isozyme per ml. Hydroxylation of lauric acid 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 $NADP^+$, 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. Hoch, U., et al., Structural determination of the substrate specificities and regioselectivities fo the rat and human fatty acid omega-hydroxylases, Arch. Biochem. Biophys., **373**, 63-71 (2000).
4. Guengrich, F.P. Cytochrome P450: Structure, Mechanism and Biochemistry (2nd Edition), Chapter 14. Ortiz de Montellano, P.R. (ed.) Plenum Press, (New York, NY: 1995).

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