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ProductInformation

CYTOCHROME P450 CYP4F3A ISOZYME
Human, Recombinant
Microsomes with Cytochrome P450 Reductase and Cytochrome b_5

Product Number **C 5110**
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, human cytochrome P450 reductase, and human cytochrome b_5.

Cytochrome P450 enzymes are a superfamily of heme containing monooxygenases that in humans are involved with 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 CYP4F3A isozyme metabolizes the ω-oxidation of leukotriene B_4 in humans to the less biologically active 20-hydroxyl product. It is a non-hepatic isozyme found in the endoplasmic reticulum of polymorphonuclear leukocytes.

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

The product is supplied as 0.25 nmole of cytochrome P450 isozyme in 0.5 ml of 100 mM potassium phosphate buffer, pH 7.4. Protein content, cytochrome b_5 content, cytochrome c reductase activity, and leukotriene B_4 20-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

Leukotriene B_4 20-Hydroxylase Activity:
A 0.1 ml reaction containing the following was incubated at 37 °C for 5 minutes:
1 pmole 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
30 µM leukotriene B_4
100 mM phosphate, pH 7.4

The reaction was stopped with 25 µl of 94% acetonitrile/6% glacial acetic acid and centrifuged (10,000 x *g*) for 3 minutes. A 50 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 20 minute gradient from 0-100% Phase B was used at a flow rate of 1.0 ml per minute. Mobile Phase A: 30% acetonitrile with 1 mM perchloric acid in water and Mobile Phase B: 70% methanol. The product was detected by measuring absorbance at 270 nm and comparison was made to a standard curve for 20-hydroxyleukotriene B_4 (Product No. H 4895).

Notes:
With respect to enzyme concentration, catalysis is linear up to at least 8 pmoles of cytochrome P450 isozyme per ml. Hydroxylation of leukotriene B_4 is approximately linear for 60 minutes. 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. 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).
4. Christmas, P., et al., Expression of the CYP4F3 gene. tissue-specific splicing and alternative promoters generate high and low K(m) forms of leukotriene B(4) omega-hydroxylase, J. Biol. Chem., **274**, 21191-21199 (1999).

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