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ProductInformation

Cytochrome P450 CYP2E1 Isozyme
Human, Recombinant
Microsomes with Cytochrome P450 Reductase and Cytochrome b_5

Product Code **C 5740**
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 cytochrome b_5. The recombinant CYP2E1 has the same mobility (Western immunoblotting) as CYP2E1 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 and human CYP2E1 has a molecular weight of approximately 57 kDa.

CYP2E1 activates low molecular weight carcinogenic compounds, such as benzene and urethane. Substrates also include chlorzoxazone, halothane, aniline, theophylline, and N,N-dimethylformamide. Common inhibitors of the 2E1 isozyme are pyridine, diethyldithiocarbamate, and disulfiram. Human CYP2E1 activity is inhibited by low concentrations (0.001%) of common organic solvents, such as ethanol, acetone, DMSO, and acetone.

The product contains 1.0 nmole of cytochrome P450 isozyme in 100 mM potassium phosphate, pH 7.4. A substantial amount of apoprotein is detected. Protein content, cytochrome c reductase activity, and 4-nitrophenol hydroxylase activity of the microsomes are reported on a lot-to-lot basis.

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, is stable for at least 18 months. For prolonged storage, freeze in working aliquots at –70 °C. Avoid repeated freezing and thawing.

Procedure

4-Nitrophenol Hydroxylase Activity

A 0.5 ml reaction containing the following was incubated at 37 °C for 30 minutes:
50 pmoles of 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
0.5 mM 4-nitrophenol
50 mM potassium phosphate, pH 7.4
A blank reaction was performed using the same reaction mixture with the addition of the cytochrome P450 isozyme immediately before stopping the reaction.

The reaction was stopped by the addition of 0.1 ml of 20% trichloroacetic acid and centrifuged at 10,000 x *g* for 1 minute. 500 µl of the supernatant was mixed with 0.25 ml of 2 N NaOH and the absorbance measured at 535 nm (using water as the reference). The measured absorbance was corrected by subtracting the value of the blank reaction. The amount of product formed was determined by comparing the corrected absorbance value to the absorbance of the product (4-nitrocatechol) under the same conditions.

Notes: With respect to enzyme concentration, catalysis is linear up to at least 160 pmoles of cytochrome P450 isozyme per ml. Hydroxylation of 4-nitrophenol 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).
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