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ProductInformation

Cytochrome P450 CYP1A2 Isozyme
Human, Recombinant
Microsomes with Cytochrome P450 Reductase

Product Number **C 5614**
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 CYP1A2 has the same mobility (Western immunoblotting) as CYP1A2 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 CYP1A2 has a molecular weight of approximately 58 kDa.

Substrates also include caffeine, clozapine, estradiol, ethoxyresorufin, phenacetin, verapamil, and warfarin. CYP1A2 is a major pathway for the 6-hydroxylation of melatonin *in vivo* and for the 5-hydroxylation of thiabendazole. Identified inhibitors include amiodarone, furafylline, and cimetidine. Tobacco, insulin, and omeprazole are among those compounds identified as inducers.

The product contains 0.5 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 phenacetin deethylase 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

Phenacetin Deethylase Activity

A 0.5 ml reaction containing the following was incubated at 37 °C for 10 minutes:
10 pmole 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
0.2 mM phenacetin (dissolved in methanol, final methanol concentration in assay is 1%)
100 mM potassium phosphate, pH 7.4,

The reaction was stopped with 50 µl of acetonitrile and centrifuged at 10,000 x *g* for 3 minutes. 50 µl of the supernatant was injected into a 4.6 x 250 mm, 5µ, C18 HPLC column and separated at 45 °C with a flow rate of 1.0 ml per minute. The mobile phase was initially 10% methanol increasing to 25% methanol over 6 minutes. The product was detected by its absorbance at 244 nm and quantitated by comparing to the absorbance of a standard curve for acetomidophenol.

References

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LCM/MAM 3/04