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ProductInformation

CYTOCHROME P450 CYP3A7 ISOZYME
Human, Recombinant
Microsomes with Cytochrome P450 Reductase and Cytochrome b_5

Product Number **C 3610**
Storage Temperature –70 °C

Product Description

The microsomal product is prepared from insect cells (*Sf* 9) infected with recombinant baculovirus containing cDNA inserts for the human cytochrome P450 isozyme, human cytochrome P450 reductase, and human cytochrome b_5. The recombinant CYP3A7 has the same mobility (western immunoblotting) as CYP3A7 in human liver microsomes.

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 CYP3A7 isozyme is found primary in the fetal liver and adult kidney.

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 potassium phosphate buffer, pH 7.4. A substantial amount of apoprotein is detected. Protein content, cytochrome b_5 content, cytochrome c reductase activity, and testosterone 6β-hydroxylase activity of the microsomes are reported on a lot-to-lot basis.

Precautions and Disclaimer

In general, ≤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.

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

Testosterone 6β-Hydroxylase Activity:
A 0.5 ml reaction containing the following was incubated at 37 °C for 10 minutes:
50 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
0.2 mM testosterone
100 mM phosphate, pH 7.4
The reaction was stopped with 250 µl of acetonitrile 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 8 minute gradient from 58% methanol to 62% methanol was used at a flow rate of 1.0 ml per minute. The product was detected by measuring absorbance at 254 nm and comparison was made to a standard curve for 6β-hydroxytestosterone (Product No. H 2898).

Notes:
With respect to enzyme concentration, catalysis is linear up to at least 200 pmoles of cytochrome P450 isozyme per ml. Hydroxylation of testosterone 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. Ohmori, S., et al., Steroid hydroxylation by human fetal CYP3A7 and human NADPH-cytochrome P450 Reductase coexpressed in insect cells using baculovirus. Res. Commun. Mol. Pathol. Pharmacol., **100**, 15–28 (1998).
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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