3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

CYTOCHROME P450 CYP1A1 ISOZYME
Human, Recombinant
Microsomes with Cytochrome P450 Reductase

Product Number **C 3735**
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.

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 potassium phosphate, pH 7.4. A substantial amount of apoprotein is detected. Protein content, cytochrome c reductase activity, and 7-ethoxyresorufin O-deethylase 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

7-Ethoxyresorufin O-Deethylase Activity:
A 2.0 ml reaction containing the following was incubated at 37 °C:
2.5 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
1 µg/ml 7-ethoxyresorufin
100 mM potassium phosphate, pH 7.4
Fluorescence of the resorufin product is measured continously (excitation at 550 nm and emission at 586 nm) in a spectrofluorometer. The increase in fluorescence intensity obtained in the linear portion of the curve is compared to a standard curve for resorufin (Product No. R 3257)

Notes: With respect to enzyme concentration, catalysis is linear up to at least 100 pmoles of cytochrome P450 isozyme per ml. Hydroxylation of 7-ethoxyresorufin is approximately linear for 20 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. Bofinger, D. P., et al., Effect of TCDD Exposure on CYP1A1 and CYP1B1 Expression in Explant Cultures of Human Endometrium, Toxicol. Sci., **62**, 299-314 (2001).
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).

KAA/MAM 1/02