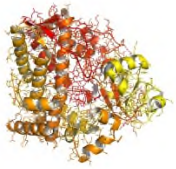
CYP450-GP



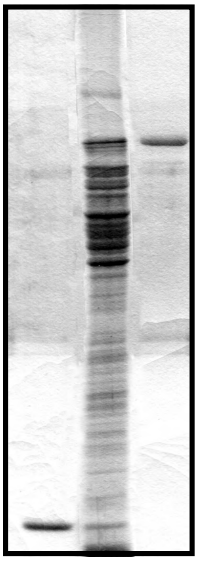
PRODUCT NUMBER Hu-P0010
HUMAN LIVER CYTOCHROME B_5
Enzyme Purified from Human Liver Microsomes
LOT P6

B_5 CONTENT =	**41.9 nmol/ml**
PROTEIN CONTENT =	**1.1 mg/ml**
SPECIFIC CONTENT =	**38.1 nmol/mg protein**

Cytochrome b_5 (b_5) was isolated from hepatic microsomes derived from a single donor liver sample using conventional purification techniques, including hydrophobic, anion-exchange, and hydroxylapatite adsorption chromatographies. Human b_5 is provided in 100 mM potassium phosphate buffer (pH 7.4) containing 0.1 mM EDTA, 0.1 mM DTT, and 20% glycerol.

♦ Purity

Purity has been determined by electrophoresis on 10% acrylamide gels run with a discontinuous buffer system. Human b_5 migrates as a single band with a molecular weight of 17.5 kDa (see Fig. 1, lane A). B_5 content was measured from the the absolute oxidized spectrum using an extinction coefficient (E) of 117 mM^{-1} cm^{-1} at 413 nm.



A B C

SDS-PAGE analysis of purified human liver cytochrome b_5 .
Lane A, **cytochrome b_5** (0.5 µg);
Lane B, liver microsomes (10 µg);
Lane C, P450 Reductase (0.5 µg);

♦ Reconstitution

Addition of b_5 to a P450 reconstituted system (containing P450 enzyme, P450 reductase, and phospholipid) often results in metabolic properties (e.g., K_M) more closely resembling those of intact liver microsomes. This is especially true with CYP2E1, which seems to require b_5 for efficient catalytic function. B_5 should be added to the P450 reconstituted system at a molar ratio of at least 4:1 (200 pmol b_5 : 50 pmol P450).

♦ Storage

Cytochrome b_5 should be stored @ -80°C. Avoid repeated freeze-thawing cycles.

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