

CYP450-GP



PRODUCT NUMBER Hu-P001 HUMAN LIVER CYP2A6

P450 Enzyme Purified from Human Liver Microsomes

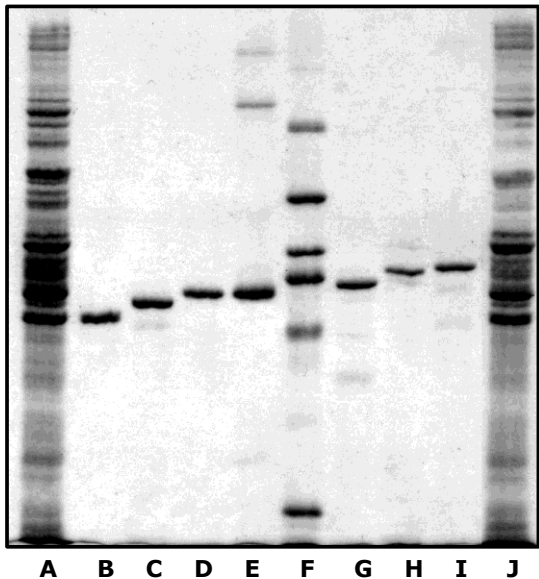
LOT #3

P450 CONTENT = **3.9 nmol/ml**
PROTEIN CONTENT = **0.6 mg/ml**
SPECIFIC CONTENT = **6.1 nmol P450/mg protein**

CYP2A6 was purified from liver microsomes from a single human subject using conventional techniques, including hydrophobic, anion-exchange, and hydroxylapatite adsorption chromatographies. Human CYP2A6 is provided in a solution containing 100 mM potassium phosphate buffer (pH 7.4), 0.1 mM EDTA, 0.1 mM DTT, and 20% glycerol.

◆ Purity

Purity has been determined by electrophoresis on 7.5% acrylamide gels run with the discontinuous buffer system. CYP2A6 migrates as a single band with a molecular weight of 49 kDa (see Fig. 1, lane C). CYP2A6 is a low-spin heme protein when oxidized with a ferrous carbonyl Soret maximum at 451 nm.



SDS-PAGE analysis of purified human liver P450 enzymes.

Lanes A & J, human liver microsomes (10 µg)

Lane B, CYP2D6 (0.5 µg)

LANE C, CYP2A6 (0.5 µg)

Lane D, CYP3A4 (0.5 µg)

Lane E, CYP2C8 (0.5 µg)

Lane F, Molecular Weight Standards (0.5 µg each)

Lane G, CYP4A11 (0.5 µg)

Lane H, CYP2E1 (0.5 µg)

Lane I, CYP2C9 (0.5 µg)

◆ Reconstitution

CYP2A6 catalytic activity is assessed upon reconstitution of the enzyme with NADPH:P450 reductase, synthetic dilauroylphosphatidyl-choline and, *importantly*, cytochrome b₅. A reconstituted system containing 50 pmol CYP2A6, 150 pmol human liver P450 reductase, and 15 µg phospholipid exhibits a turnover number of 3.1 min⁻¹ with coumarin as substrate; in the presence of 200 pmol human liver b₅, coumarin 7-hydroxylase activity increases to 11.1 min⁻¹.

◆ Storage - CYP2A6 should be stored @ -80°C. Avoid repeated freeze-thawing cycles.