

CYP450-GP



PRODUCT NUMBER Hu-P003 HUMAN LIVER CYP2C9

P450 Enzyme Purified from Human Liver Microsomes

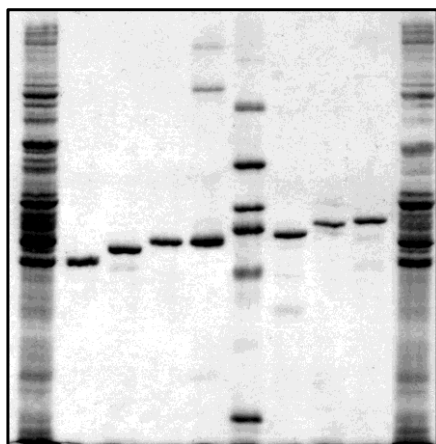
LOT #11

P450 CONTENT = 15.5 nmol/ml
PROTEIN CONTENT = 1.35 mg/ml
SPECIFIC CONTENT = 11.5 nmol P450/mg protein

CYP2C9 was purified from liver microsomes from a single human subject using conventional techniques, including hydrophobic, anion-exchange, and hydroxylapatite adsorption chromatographies. Human CYP2C9 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. CYP2C9 migrates as a single band with a molecular weight of 56 kDa (see Fig. 1, lane I). CYP2C9 is a low-spin heme protein when oxidized, and has a ferrous carbonyl Soret maximum at 451 nm.



SDS-PAGE analysis of purified human liver P450 enzymes.

Lanes A & J, 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

CYP2C9 activity is assessed upon the enzyme's reconstitution with NADPH:P450 reductase, synthetic dilauroylphosphatidyl-choline and, depending upon the substrate, cytochrome b₅. A reconstituted system containing 50 pmol CYP2C9, 150 pmol human liver P450 reductase, and 15 µg phospholipid exhibits a turnover number of 2.7 min⁻¹ with tolbutamide; inclusion of 200 pmol human liver b₅ increases this turnover number to 4.3 min⁻¹. Full reconstitution details are given in an accompanying instruction sheet.

◆ Storage

CYP2C9 should be stored @ -80°C. Avoid repeated freeze-thawing cycles.