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 hemeprotein 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 dilauroylphosphatidylcholine 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.