# CYP450-GP



## PRODUCT NUMBER Hu-P005 HUMAN LIVER CYP4A11

P450 Enzyme Purified from Human Liver Microsomes LOT #5

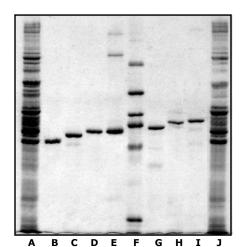
P450 CONTENT = 16.4 nmol/ml PROTEIN CONTENT = 1.5 mg/ml

SPECIFIC CONTENT = 10.9 nmol P450/mg protein

CYP4A11 was purified from liver microsomes from a single human subject using conventional techniques, including hydrophobic, anion-exchange, and hydroxylapatite adsorption chromatographies. Human CYP4A11 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.

## ♦ <u>Purity</u>

Purity has been determined by electrophoresis on 7.5% acrylamide gels run with the discontinuous buffer system. CYP4A11 migrates as a single band with a molecular weight of 53 kDa (see Fig. 1, lane G). CYP4A11 is a low-spin hemeprotein 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

CYP4A11 catalytic activity is assessed upon reconstitution of the enzyme with NADPH:P450 reductase, synthetic dilauroylphospha-

tidlycholine and, <u>importantly</u>, cytochrome b<sub>5</sub>. A reconstituted system containing 50 pmol CYP4A11, 150 pmol human liver P450 reductase, and 15 µg phospholipid exhibits a turnover number of 16.1 min<sup>-1</sup> with laurate as substrate; in the presence of 200 pmol human liver b<sub>5</sub>, laurate 12-hydroxylase activity increases to 45.7 min<sup>-1</sup>. Full details for reconstitution are given in an accompanying instruction sheet.

♦ **Storage** CYP4A11 should be stored @ -80°C. Avoid repeated freeze-thawing cycles.