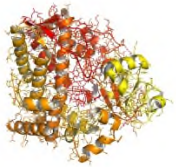
CYP450-GP



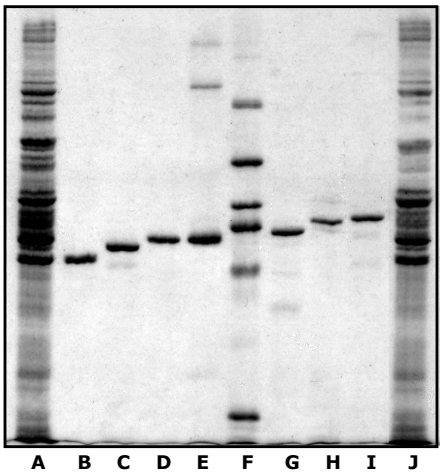
PRODUCT NUMBER Hu-P006
HUMAN LIVER CYP3A4
P450 Enzyme Purified from Human Liver Microsomes
LOT #3

P450 CONTENT =	**16.8 nmol/ml**
PROTEIN CONTENT =	**1.2 mg/ml**
SPECIFIC CONTENT =	**14.0 nmol P450/mg protein**

CYP3A4 was purified from liver microsomes from a single human subject using conventional techniques, including hydrophobic, anion-exchange, and hydroxylapatite adsorption chromatographies. Human CYP3A4 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. CYP3A4 migrates as a single band with a molecular weight of 51.5 kDa (see Fig. 1, lane D). CYP3A4 is a low-spin hemeprotein when oxidized with a ferrous carbonyl Soret maximum at 450 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

In contrast to other human P450 enzymes, the catalytic activity of purified CYP3A4 can be difficult to reconstitute. Success requires reconstitution of 50 pmol CYP3A4 with 150 pmol human liver P450 reductase, 200 pmol cytochrome b_5, and 20 µg of a phospholipid mixture comprised of synthetic dilauroylphosphatidlycholine, dioleoylphosphatidylcholine, and dilauroylphosphatidylserine (1:1:1 ratio). The addition of CHAPS (100 µg/ml) and/or glutathione (3 mM) may enhance CYP3A4-mediated metabolism, depending upon the particular substrate. Full details for reconstitution are given in an accompanying instruction sheet.

♦ Storage

CYP3A4 should be stored @ -80°C. Avoid repeated freeze-thawing cycles.

CYP450-GP, 2870 Scott Street Suite 103, Vista, CA 92081
Phone 760.295.7426; Fax 760.418.6472; sales@cyp450-gp.com