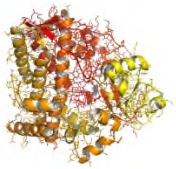
CYP450-GP



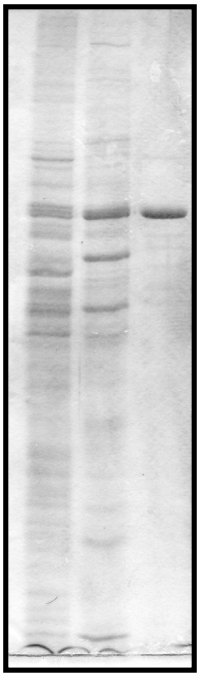
PRODUCT NUMBER Hu-P008
HUMAN LIVER NADPH:P450 REDUCTASE
Enzyme Purified from Human Liver Microsomes
LOT UP879

ENZYME ACTIVITY =	**37.0 nmol (124,800 units)/ml**
PROTEIN CONTENT =	**4.9 mg/ml**
SPECIFIC ACTIVITY =	**7.6 nmol (25,470 units)/mg protein**

NADPH:P450 oxidoreductase (P450 reductase) was purified from liver microsomes from a single human subject using conventional techniques, including hydrophobic, anion-exchange, affinity, and hydroxylapatite adsorption chromatographies. Human P450 reductase is provided in 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 10% acrylamide gels run with the discontinuous buffer system. P450 reductase migrates as a single band with a molecular weight of 75 kDa (see Fig. 1, lane C). One unit P450 reductase activity is defined as that amount catalyzing reduction of 1 nmol ferricytochrome c/min at 30°C in 300 mM potassium phosphate buffer (pH 7.7). One nmol P450 reductase contains 3,370 units of enzyme activity.



A B C

SDS-PAGE analysis of purified human liver P450 reductase.
Lane A, cytochrome b_5 (0.5 µg);
Lane B, liver microsomes (10 µg);
Lane C, **P450 Reductase** (0.5 µg);

♦ Reconstitution

Human P450 reductase serves, in combination with phospholipid, to reconstitute purified P450 enzyme activity. The molar ratio of P450 reductase to P450 enzyme used should be at least 3**:**1 [e.g., 150 pmol (ca 500 units) P450 reductase**:**50 pmol P450]. P450 reductase is allowed to reconstitute with a given P450 enzyme for 3 min at 37°C in the presence of phospholipid. Full details for reconstitution are given in an accompanying instruction sheet.

♦ **Storage** P450 reductase should be stored @ -80°C. Avoid repeated freeze-thawing.

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