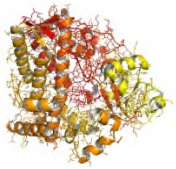
CYP450-GP



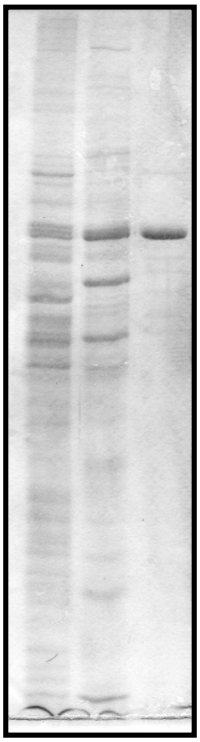
PRODUCT NUMBER Hu-P008
HUMAN LIVER NADPH:P450 OXIDOREDUCTASE
Enzyme Purified from Human Liver Microsomes
LOT UP879

ENZYME ACTIVITY =	**19.1 nmol (64,200 units)/ml**
PROTEIN CONTENT =	**4.9 mg/ml**
SPECIFIC ACTIVITY =	**3.9 nmol (13,100 units)/mg protein**

NADPH:P450 oxidoreductase (i.e., 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. 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 22°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 Oxidoreductase.
Lane A, liver microsomes (10 µg);
Lane B, DEAE cellulose chromatography eluate (10 µg);
Lane C, **Purified P450 Oxidoreductase** (0.5 µg);

♦ Reconstitution

Human P450 reductase serves, in combination with phospholipid, to reconstitute the activity of purified P450 enzymes. The molar ratio of P450 reductase to P450 enzyme used in the reconstituted system should be at least 3**:**1 [e.g., 150 pmol (ca 500 units) P450 reductase**:**50 pmol P450]. P450 reductase should be 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 the *Protocols* section of the CYP450-GP website.

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

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