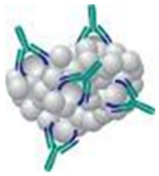
CYP450-GP



PRODUCT Hu-A005

ANTI-HUMAN CYP3A4 IgG

Polyclonal Antibody Developed in Rabbits, IgG Fraction

LOT RaQ/B#3-7

Antiserum was developed in rabbits using purified human liver CYP3A4 as immunogen. The whole IgG fraction was purified from antiserum using caprylic acid/ammonium sulfate fractionation. Anti-human CYP3A4 IgG is provided as a powder after lyophylization from 100 mM potassium phosphate buffer (pH 7.4), 150 mM KCl, and 2.5 µM thimerosal (added as a preservative).

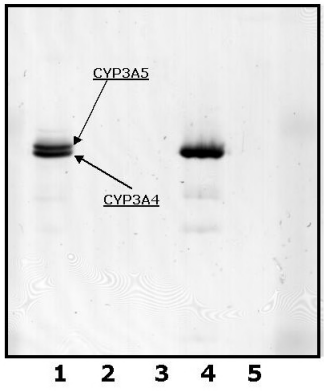
♦ Specificity and Purity

Specificity has been determined by Western blotting. Anti-human CYP3A4 IgG reacts primarily with CYP3A4 (51.5 kDa) but also recognizes CYP3A5 (52.5 kDa) and CYP3A7 (51.5 kDa) in human liver microsomes. Cross-reaction of the antibody with homologous CYP3A proteins in animal liver microsomes has not been determined nor has specificity with whole liver homogenates or S-9 fractions

Antibody purity has been established by SDS-PAGE run under denaturing conditions. Two protein bands with molecular weights of 50 kDa and 25 kDa can be visualized by Coomassie blue staining, which correspond to the heavy and light chains, respectively, of rabbit IgG.

♦ Reconstitution of Lyophylized Product and Storage

Store lyophylized product at 0-5°C. For Western blotting, reconstitute IgG to 1 mg protein/ml final concentration by adding appropriate amount of PBS/50% glycerol to the vial of lyophylized IgG and mixing gently until powder dissolves. Solution can then be stored at -20°C, as the presence of 50% glycerol will prevent freeze/thawing. For immunoinhibition studies, reconstitute anti-CYP2D6 IgG in an appropriate buffer (e.g., 100 mM potassium phosphate, pH 7.4) to a concentration of 10-20 mg IgG/ml, and also store at -20°C. In the absence of glycerol, however, the number of freeze/thaw cycles should be kept to a minimum.



Immunoreactivity of Anti-CYP3A4 IgG with human liver proteins

Lane 1 = UC9209 liver microsomes (5 µg)
Lane 2 = Purified CYP4A11 (0.1 µg)
Lane 3 = Purified CYP4F2 (0.1 µg)
Lane 4 = Purified CYP3A4 (0.1 µg)
Lane 5 = HK31 kidney microsomes (10 µg)
Arrows indicate relative migration of CYP3A4 and CYP3A5

♦ Use for Western Blotting

Incubate blots overnight with 2.5 - 5.0 µg rabbit anti-human CYP3A4 IgG/ml of appropriate blocking solution. After washing to remove unbound CYP3A4 antibody, incubate with an anti-rabbit IgG conjugate of choice (e.g, anti-rabbit IgG-peroxidase or anti-rabbit IgG-biotin), and develop accordingly. A detailed Western blotting method can be found in the Protocols section.

♦ Use for Immunoinhibition

Incubation of anti-human CYP3A4 IgG with human liver microsomes at a ratio of 2 mg IgG/mg IgG/mg microsomal protein (5 mg IgG/nmol microsomal P450) before reaction initiation will typically give 80-90% inhibition of an exemplary CYP3A4-catalyzed reaction (e.g., nifedipine oxidation; see attached). Methodology for conducting P450 immunoinhibition assays is given in the Protocols section.

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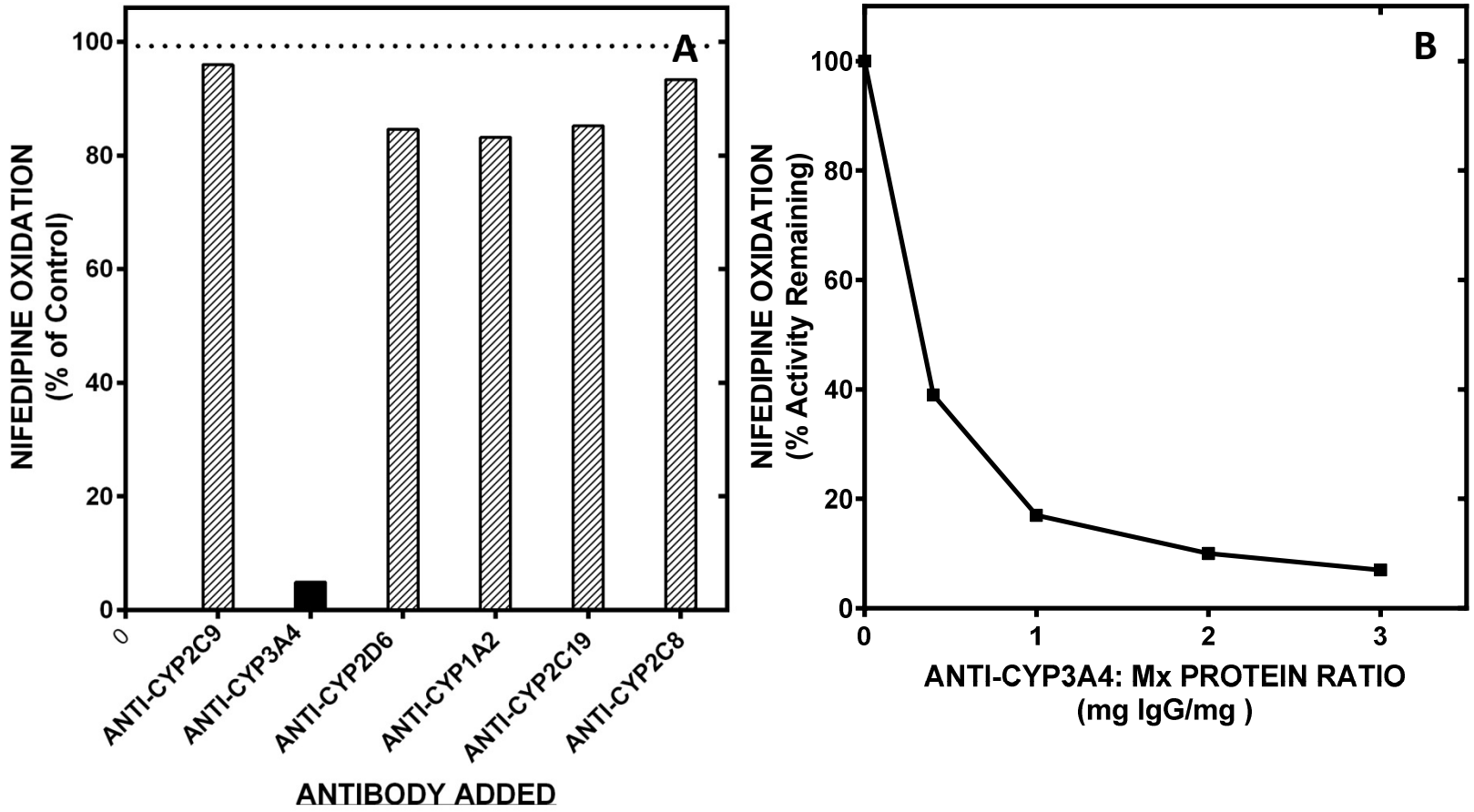
SPECIFIC INHIBITION OF NIFEDIPINE OXIDATION IN HUMAN LIVER MICROSOMES BY ANTI-CYP3A4



Panel A - Antibodies to human CYP3A4 elicited marked inhibition (95% at 2 mg IgG/mg microsomal protein) of nifedipine oxidation by human liver microsomes (pool of 50) whereas the other P450 antibodies examined had negligible effects on this CYP3A4-catalyzed reaction. **Panel B** - Maximal inhibition (90%) of nifedipine oxidation by the same liver microsomes was achieved using an anti-CYP3A4 IgG:mx protein ratio of 3.0 mg/mg. Control rates (+ preimmune IgG) of nifedipine metabolism were 2.36 nmol nifedipine pyridine metabolite formed/min/mg protein.