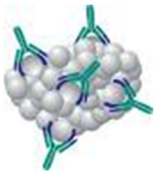
CYP450-GP



PRODUCT Hu-A007

ANTI-HUMAN CYP4A11 IgG

Polyclonal Antibody Developed in Rabbits, IgG Fraction

LOT RaR/B#3-7

Antiserum was developed in rabbits using purified human liver CYP4A11 as immunogen. The whole IgG fraction was purified from antiserum using caprylic acid/ammonium sulfate fractionation. Anti-human CYP4A11 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).

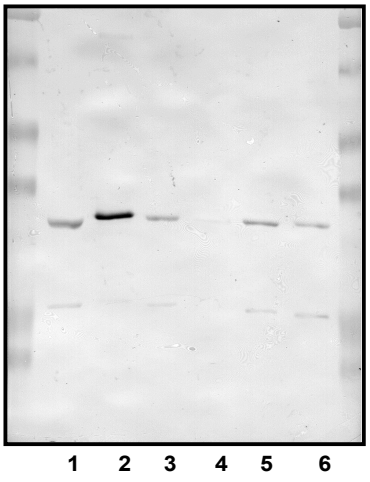
♦ Specificity and Purity

Specificity has been determined by Western blotting. Anti-human CYP4A11 IgG reacts exclusively with its corresponding 53 kDa antigen in human liver microsomes. In addition, the antibody recognizes the homologous CYP4A proteins in rat liver microsomes.

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, anti-CYP4A11 IgG should be reconstituted to 1 mg protein/ml by adding the appropriate amount of PBS/50% glycerol to the vial, and and mixing gently until dissolved. The solution can then be stored at -20°C, as the presence of 50% glycerol prevents freeze/thawing. For immunoinhibition studies, reconstitute anti-CYP4A11 IgG in an appropriate buffer (e.g., 100 mM potassium phosphate, pH 7.4) at a concentration of 10-20 mg IgG/ml, and store at -20°C. The number of freeze/thaw cycles should be kept to a minimum.



Immunoreactivity of Anti-CYP4A11 IgG with human liver proteins

Lane 1 = Liver microsomes from Subject A (15 µg)
Lane 2 = Purified CYP4A11 (0.1 µg)
Lane 3 = Liver microsomes from Subject B (15 µg)
Lane 4 = Purified CYP2E1 (0.1 µg)
Lane 5 = Liver microsomes from Subject C (15 µg)
Lane 6 = Liver microsomes from Subject D (15 µg)

♦ Use for Western Blotting

Incubate blots overnight with 2.5 - 5.0 µg rabbit anti-human CYP4A11 IgG/ml of appropriate blocking solution. After washing to remove unbound antibody, incubate with an anti-rabbit IgG conjugate of choice (e.g, anti-rabbit IgG-peroxidase), and develop accordingly. A detailed Western blotting method can be found in the PROTOCOLS section.

♦ Use for Immunoinhibition

Incubation of anti-human CYP4A11 IgG with human liver microsomes at a ratio of 5 mg IgG/nmol microsomal P450 (1.7 mg IgG/mg microsomal protein) before reaction initiation will typically give 70-85% inhibition of an exemplary CYP4A11-catalyzed reaction (e.g., laurate 12-hydroxylation; **see attached**). Methodology for conducting P450 immunoinhibition assays is given in the PROTOCOLS section.

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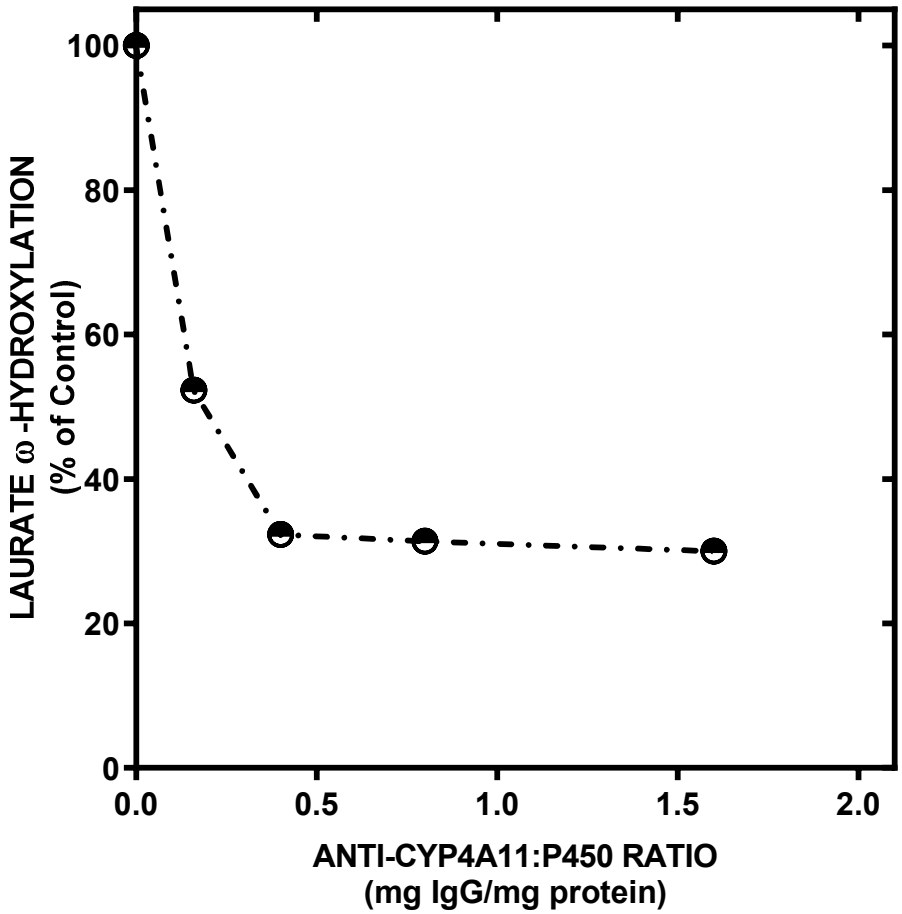
INHIBITION OF LAURATE ω -HYDROXYLATION IN HUMAN LIVER MICROSOMES BY ANTI-CYP4A11 IgG



Maximal inhibition (69%) of laurate ω-hydroxylation by liver microsomes from subject UC9603 was achieved at an anti-CYP4A11 IgG:mx protein ratio of 0.4 mg/mg. With microsomes from other human subjects, anti-CYP4A11 mediated inhibition of laurate metabolism can reach nearly 90% at similar IgG:mx protein ratios. The residual metabolite production shown here stems from the formation of 12-hydroxylaurate by P450s other than CYP4A11.