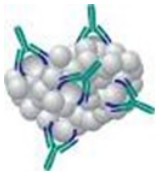
CYP450-GP



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).

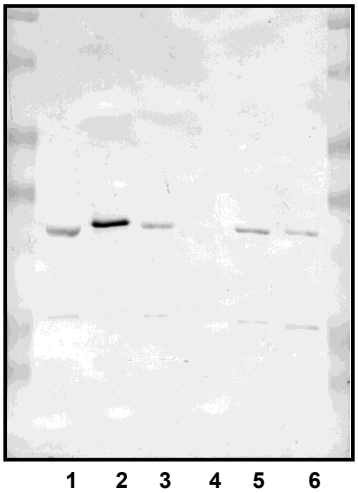
♦ Specificity and Purity

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. Specificity with whole liver homogenates or S-9 fractions has not been determined.

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 by adding 1 ml of PBS/50% glycerol to one vial of lyophilized IgG (1 mg) and mix vial gently until powder dissolves. After reconstitution, solution can be stored at -20°C, as the presence of glycerol will prevent freeze/thaw cycles. Anti-CYP4A11 IgG solutions without glycerol should be also be stored at -20°C but subjected to freeze/thaw cycles as seldom as possible.



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 CYP4A11 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 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 80-90% inhibition of an exemplary CYP4A11-catalyzed reaction (e.g., laurate 12-hydroxylation). Methodology for conducting P450 immunoinhibition assays is given in the Protocols section.