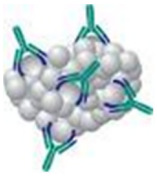
CYP450-GP



PRODUCT Hu-A008

ANTI-HUMAN CYP2C19 IgG

Polyclonal Antibody Developed in Rabbits, IgG Fraction

LOT RaY/B#3-7 M-1c

Antiserum was developed in rabbits using recombinant human CYP2C19 as immunogen. The whole IgG fraction was purified from antiserum using caprylic acid/ammonium sulfate fractionation. Anti-CYP2C19 IgG is provided in a lyophilized state after freeze-drying from 100 mM potassium phosphate buffer (pH 7.4) containing 150 mM KCl and 2.5 µM thimerosal (added as a preservative).

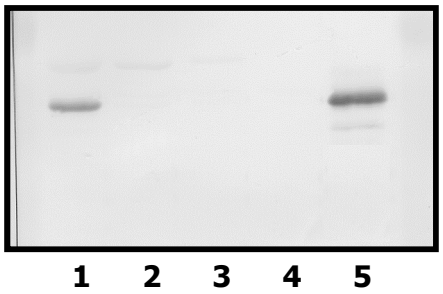
♦ Specificity and Purity

Specificity has been determined by Western blotting, where anti-human CYP2C19 IgG recognizes only its 50 kDa immunogen in human liver microsomes. Cross-reactivity with other CYP2C proteins including CYP2C8 and CYP2C9 is minimal, and was removed by solid-phase back-adsorption against these human P450s. Reactivity of anti-human CYP2C19 with animal CYP2C proteins has not been examined.

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, the IgG should be reconstituted to 1 mg protein/ml final concentration by adding the appropriate amount of PBS/50% glycerol to the vial of lyophylized IgG and mixing gently until powder dissolves. Afterwards, the solution can 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.



Reaction of Anti-CYP2C19 with human liver proteins

Lane 1 = Microsomes Subject D (CYP2C19**+**)(15 µg)
Lane 2 = Microsomes Subject N (CYP2C19**-**)(15 µg)
Lane 3 = Purified CYP2C9 (0.1 µg)
Lane 4 = Purified CYP2C8 (0.1 µg)
Lane 5 = Purified rCYP2C19 (0.1 µg)

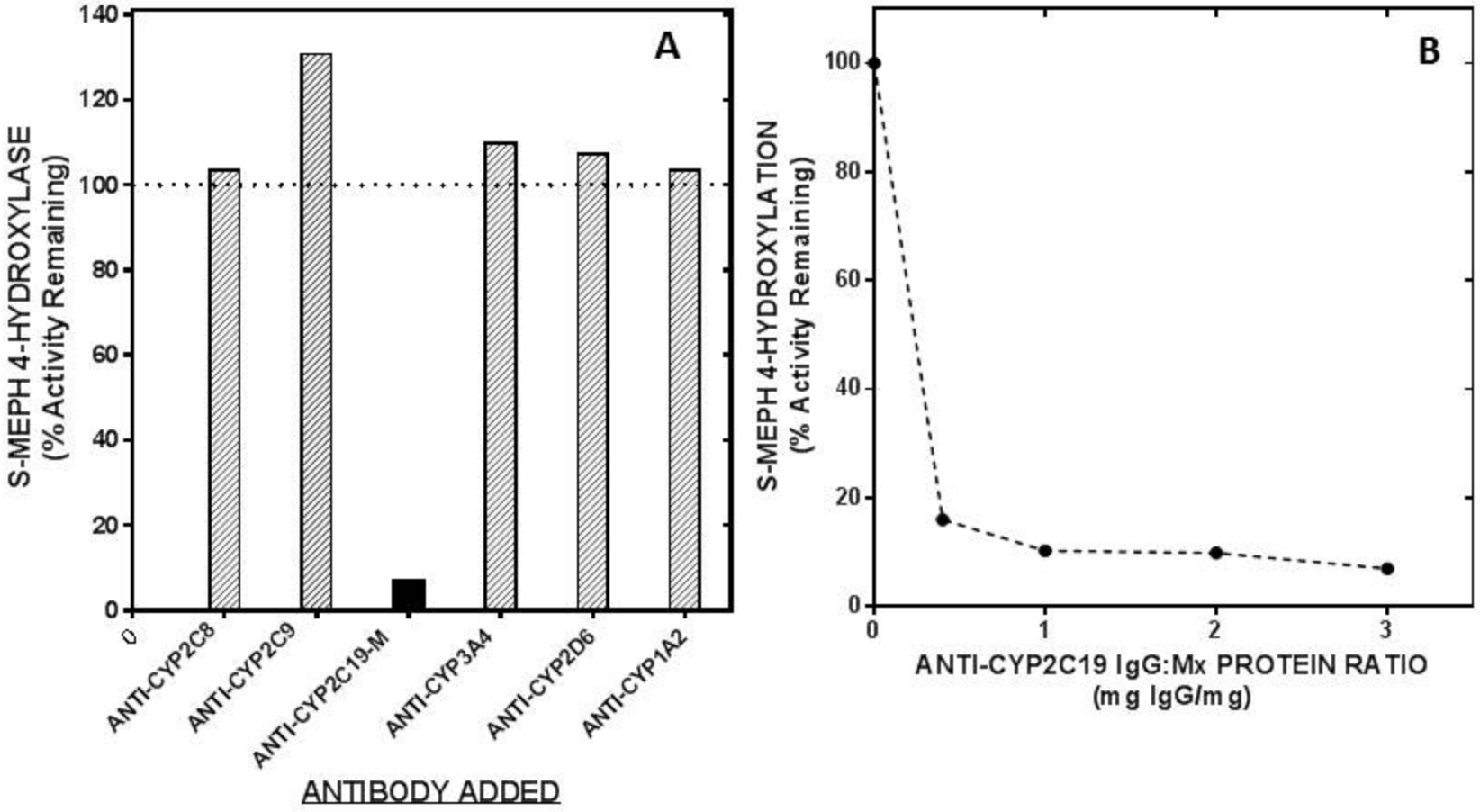
♦ Use for Western Blotting

Incubate blots overnight with 2.5 - 5.0 µg rabbit anti-human CYP2C19 IgG/ml of appropriate blocking solution. After washing to remove unbound CYP2C19 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 CYP2C19 IgG with human liver microsomes at a ratio of 1.0 mg IgG/mg microsomal protein (2.5 mg IgG/nmol microsomal P450) before reaction initiation will typically give 90% inhibition of an exemplary CYP2C19-catalyzed reaction (e.g., *S*-mephenytoin 4'-hydroxylation; **see attached**). Methodology for conducting P450 immunoinhibition assays is given in the PROTOCOLS section.

INHIBITION OF *S*-MEPHENYTOIN (*S*-MEPH) 4'-HYDROXYLATION IN HUMAN LIVER MICROSOMES BY ANTI-CYP2C19



Panel A - Antibodies to human CYP2C19 had a marked inhibitory effect (93% at 1 mg IgG/mg mx protein) on *S*-MEPH 4'-hydroxylation by human liver microsomes whereas the other P450 antibodies tested failed to decrease this CYP2C19-catalyzed reaction. Panel B – In a separate experiment, optimal inhibition (90%) of microsomal *S*-MEPH 4'-hydroxylation was achieved at an anti-CYP2C19 IgG:mx protein ratio of 1 mg/mg. Control (+preimmune IgG) rates of *S*-MEPH metabolism were 19.0 pmol 4-hydroxymephenytoin formed/min/mg protein.