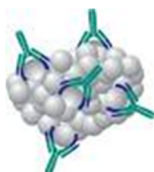


CYP450-GP



PRODUCT NUMBER 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 μ M 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 human CYP2C8 and human CYP2C9 is minimal, and was removed by solid-phase back-adsorption against these human P450s. Antibody purity has been established by SDS-PAGE run under denaturing conditions. Two protein bands with molecular weights of 50 kDa and 25 kDa are visualized upon Coomassie blue staining, which correspond to the heavy and light chains, respectively, of rabbit IgG.

◆ **Reconstitution of Lyophilized Product and Storage**

Store lyophilized product at 0-5°C. Reconstitute the IgG to 1.0 mg protein/ml final concentration by adding the appropriate amount of PBS/50% glycerol to the vial, and mix gently until the powder dissolves. The solution can then be stored at -20°C, as the presence of 50% glycerol will prevent freeze/thawing. For immunoinhibition studies, reconstitute anti-CYP2C19 IgG in an appropriate buffer (e.g., 100 mM potassium phosphate, pH 7.4) to 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 in the absence of glycerol.



1 2 3 4 5

Reaction of Anti-CYP2C19 with human liver proteins

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

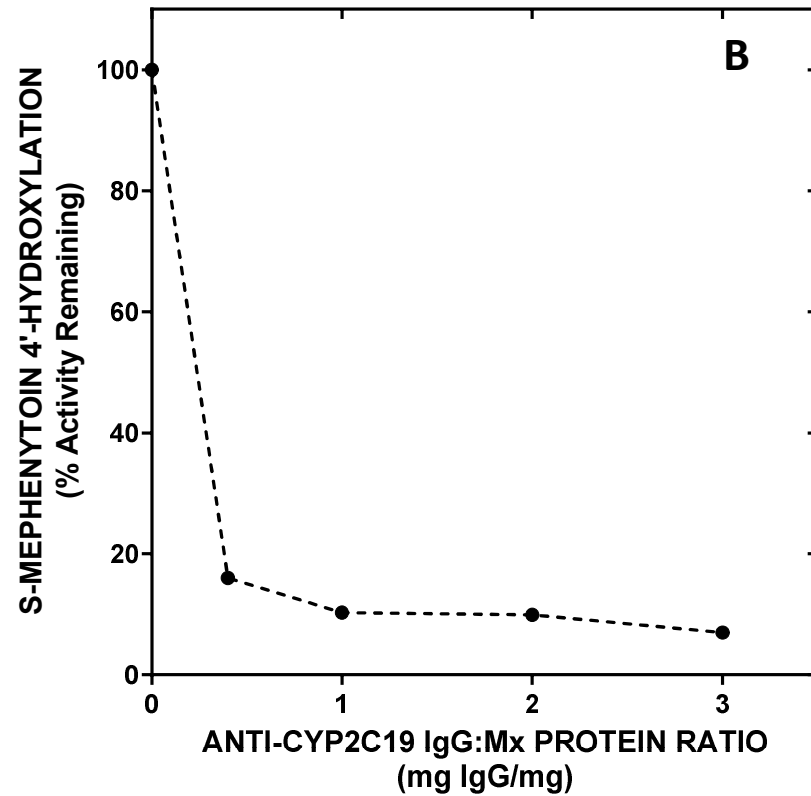
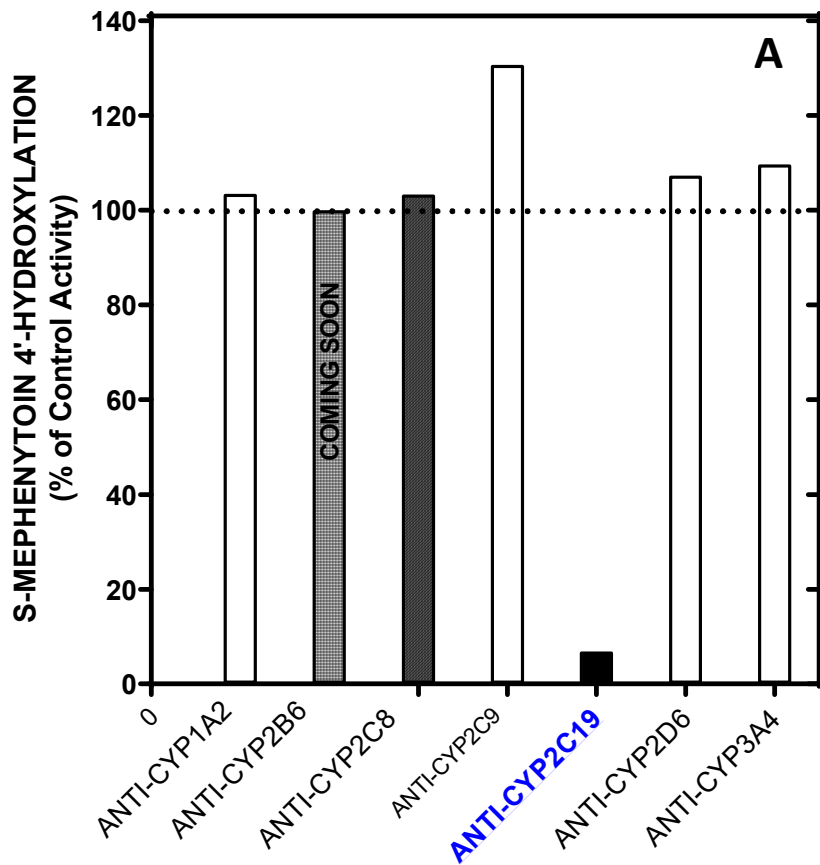
◆ **Use for Western Blotting**

Incubate blots overnight with 2.5 - 5.0 μ g 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 YP2C19-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 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*-mephenytoin 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*-mephenytoin 4'-hydroxylation was achieved at an anti-CYP2C19 IgG:mx protein ratio of 1 mg/mg. Control (+preimmune IgG) rates of *S*-mephenytoin oxidation were 19.0 pmol 4-hydroxymephenytoin formed/min/mg protein.