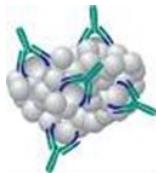


CYP450-GP



PRODUCT Hu-A003

ANTI-HUMAN CYP2C9 IgG

Polyclonal Antibody Developed in Rabbits, IgG Fraction
LOT RaJ/B#3-7

Antiserum was developed in rabbits using purified human liver CYP2C9 as immunogen. The whole IgG fraction was purified from antiserum using caprylic acid/ammonium sulfate fractionation. Anti-human CYP2C9 IgG is provided as a powder after lyophilization from 100 mM potassium phosphate buffer (pH 7.4), 150 mM KCl, and 2.5 μ M thimerosal (added as a preservative).

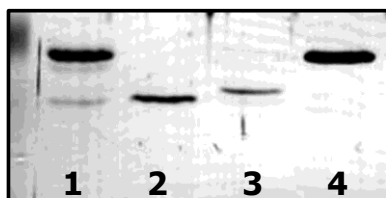
◆ **Specificity and Purity**

Specificity has been determined by Western blotting. Anti-human CYP2C9 IgG reacts primarily with CYP2C9 (56 kDa) but also recognizes CYP2C19 (50 kDa) in human liver microsomes; cross-reaction with CYP2C8 (52 kDa) is less extensive. The antibody also cross-reacts with the homologous CYP2C proteins in rat and mouse 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 Lyophilized Product and Storage**

Store lyophilized 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-CYP2C9 IgG solutions without glycerol should be also be stored at -20°C but subjected to freeze/thaw cycles as seldom as possible.



Anti-CYP2C9 IgG Reactivity with Human Liver Proteins

Lane 1 = UCSF003 microsomes (10 μ g)

Lane 2 = Purified CYP2C19 (0.1 μ g)

Lane 3 = Purified CYP2C8 (0.1 μ g)

Lane 4 = Purified CYP2C9 (0.1 μ g)

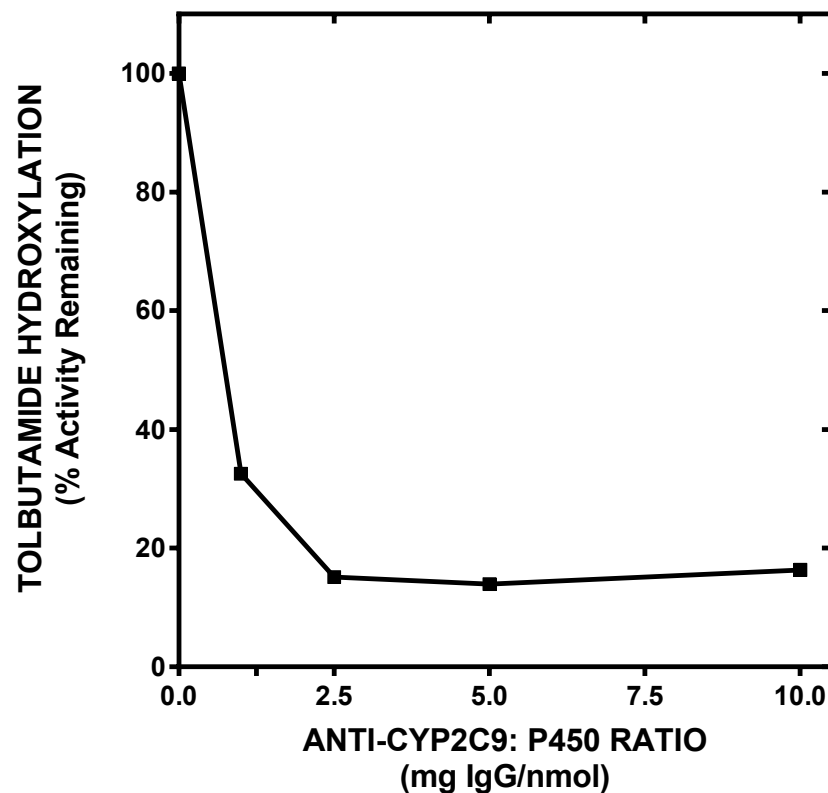
◆ **Use for Western Blotting**

Incubate blots overnight with 2.5 - 5.0 μ g rabbit anti-human CYP2C9 IgG/ml of appropriate blocking solution. After washing to remove unbound CYP2C9 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 CYP2C9 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 CYP2C9-catalyzed reaction (e.g., tolbutamide methyl hydroxylation; **see attached**). The methodology for conducting P450 immunoinhibition assays is given in the [Protocols](#) section.

INHIBITION OF TOLBUTAMIDE HYDROXYLATION IN HUMAN LIVER MICROSOMES BY ANTI-CYP2C9



Antibodies to human CYP2C9 had a marked inhibitory effect on tolbutamide methyl hydroxylation by liver microsomes from subject UC8936 whereas the other P450 antibodies tested failed to decrease this CYP2C9-catalyzed reaction (data not shown). As depicted above, maximal inhibition (86.1%) of microsomal tolbutamide hydroxylation in this human subject was achieved at an anti-CYP2C9 IgG:P450 ratio of 5.0 mg/nmol.