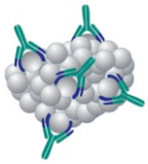
CYP450-GP



PRODUCT NUMBER Hu-A003M

ANTI-CYP2C9 IgG**

Monoclonal Antibody Developed in Mice, IgG_1 Fraction

LOT 763.15.5

Ascites fluid containing CYP2C9 monoclonal antibodies (mAb) was produced in mice upon injection with hybridomas derived from animals immunized with recombinant human CYP2C9. The IgG fraction was purified from ascites fluid using caprylic acid/ammonium sulfate fractionation. Anti-human CYP2C9 IgG (IgG_1 isotype) is provided as a powder after lyophylization from 100 mM potassium phosphate buffer (pH 7.4).

♦ Specificity and Purity

Immunospecificity has been determining by ELISA. As shown below, anti-human CYP2C9 mAb reacts only with its corresponding immunogen in human liver microsomes. Cross-reactivity with CYP2C8 and CYP2C19 is negligible. Reactivity of the monoclonal antibody with the homologous CYP2C proteins in rat and mouse liver microsomes has not been determined nor has specificity with whole human liver homogenates or S-9 fractions.

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 mouse IgG_1.

	P450 ENZYME							
ANTIBODY	**CYP1A2**	**CYP2B6**	**CYP2C8**	**CYP2C9#**	**CYP2C19**	**CYP2D6**	**CYP3A4**	**CYP3A5**
Anti-CYP2C9	**0**	**0**	**0**	**+++**	**0**	**0**	**0**	**0**

#Includes CYP2C9*1, CYP2C9*2 and CYP2C9*3 alleles.

♦ Reconstitution of Lyophylized Product and Storage

Store lyophylized product at 0-5°C. Reconstitute by adding 0.1 - 0.2 ml of an appropriate buffer (e.g., 100 mM potassium phosphate, pH 7.4) to one vial of lyophilized IgG (0.1 - 0.2 mg immunoglobulin plus 1.0 mg BSA carrier protein) and mix vial gently until powder dissolves. After reconstitution, the IgG solution can be stored at -20°C but subjected to freeze/thaw cycles as seldom as possible.

♦ Use for Western Blotting

Anti-human CYP2C9 mAb IgG does not react with its corresponding antigen (native or recombinant) on protein blots, and is therefore not suitable for Western blot analysis.

♦ Use for Immunoinhibition

Incubation of anti-human CYP2C9 mAb IgG with human liver microsomes at a ratio of 75 µg IgG/mg microsomal protein (250 µg IgG/nmol microsomal P450) will typically give 80% inhibition of an exemplary CYP2C9-catalyzed reaction (e.g., diclofenac 4'-hydroxylation; **see attached**). Methodology for conducting P450 immunoinhibition assays is given in the PROTOCOLS section.

****Crude ascites fluid containing anti-CYP2C9 mAb was supplied by NCI/NIH (NIH reference #E-077-1999/0).**

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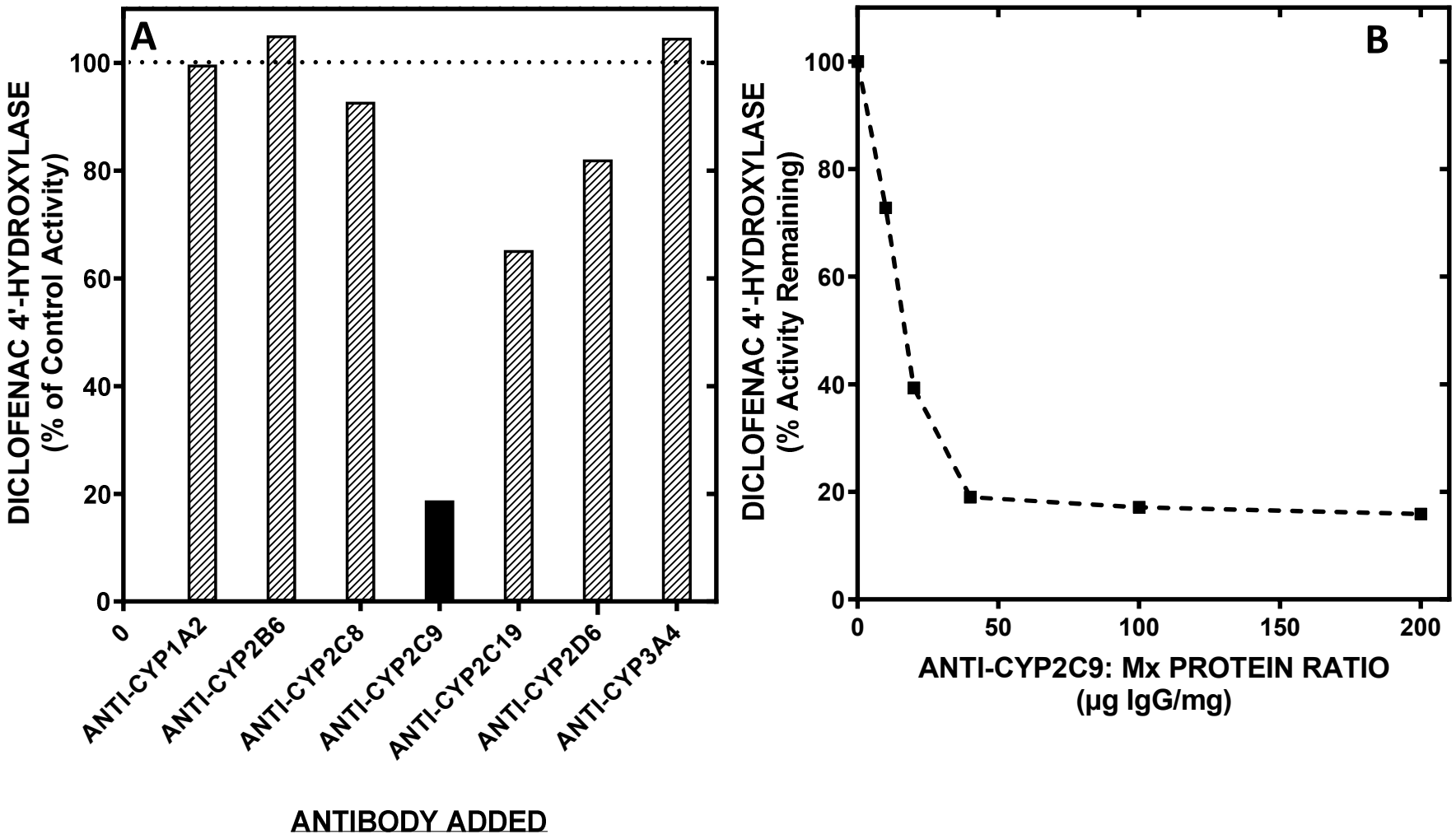
SPECIFIC INHIBITION OF DICLOFENAC 4'-HYDROXYLATION
IN HUMAN LIVER MICROSOMES BY ANTI-CYP2C9



Panel A - Anti-CYP2C9 mAb elicited a strong inhibitory effect (81% at 100 µg IgG/mg protein) on diclofenac 4'-hydroxylation by human liver microsomes whereas the other P450 antibodies tested had minor effects on this CYP2C9-catalyzed reaction. Values shown denote the average of duplicate determinations.

Panel B – In a separate experiment, maximal inhibition (83%) of microsomal diclofenac 4'-hydroxylation was achieved at an anti-CYP2C9 IgG:P450 ratio of 100 µg/mg. Control rates (+ preimmune IgG) of diclofenac 4'-hydroxylation by liver microsomes were 1.42 ± 0.4 nmol/min/mg (n = 3).