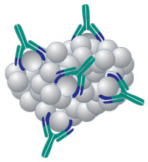
CYP450-GP



PRODUCT NUMBER Hu-A004

ANTI-HUMAN CYP2C8 IgG**

Monoclonal Antibody Developed in Mice, IgG_1 Fraction

LOT 281.1.1

Ascites fluid containing CYP2C8 monoclonal antibodies (MAb) was produced in mice upon injection with hybridomas derived from animals immunized with recombinant human CYP2C8. The whole IgG fraction was purified from ascites fluid using caprylic acid/ammonium sulfate fractionation. Anti-human CYP2C8 IgG 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 CYP2C8 MAb IgG reacts only with its corresponding immunogen in human liver microsomes. Cross-reactivity with CYP2C9 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-CYP2C8	**0**	**0**	**+++**	**0**	**0**	**0**	**0**	**0**

*Lack of immunoreactivity includes CYP2C9*1, CYP2C9*2 and CYP2C9*3

♦ 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 the 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

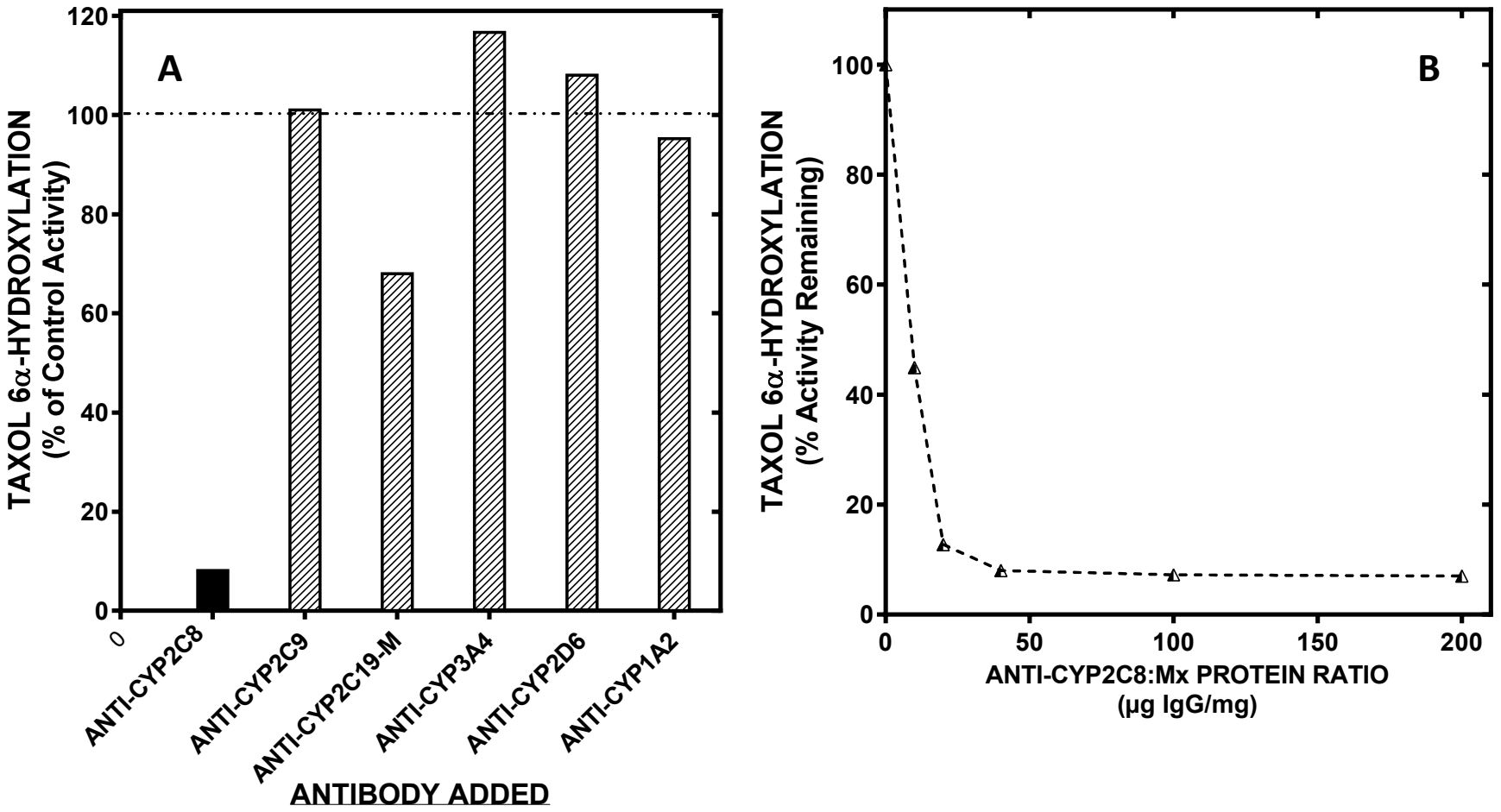
Anti-human CYP2C8 MAb IgG/ml does not react with its corresponding antigen (native or recombinant) on protein blots, and is therefore not suitable for Western blot analysis.

♦ Use for Immunoinhibition

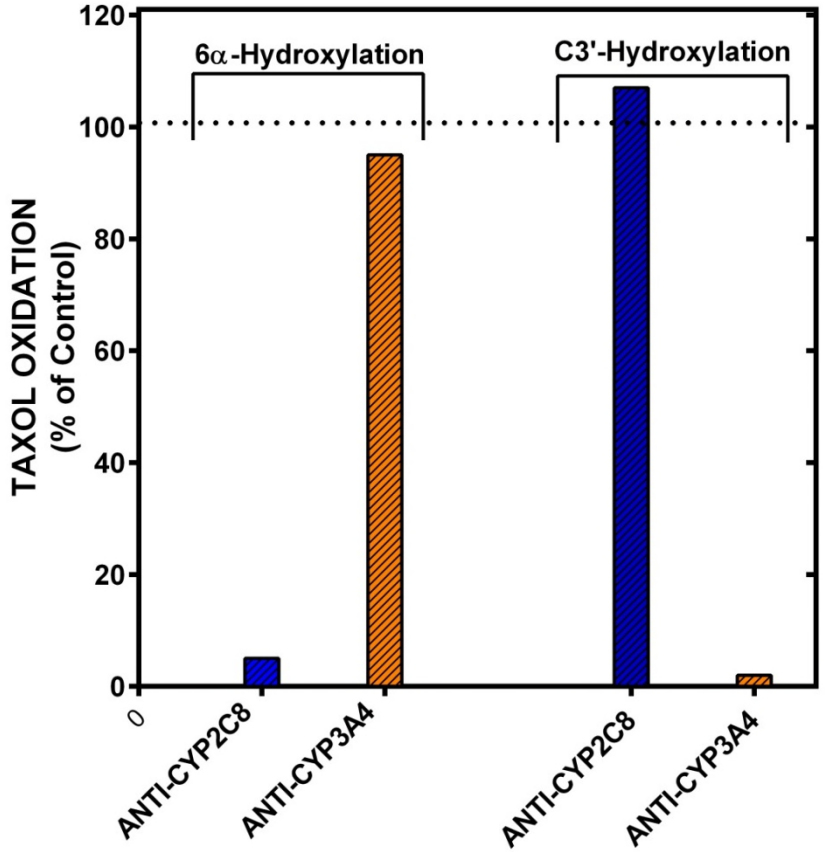
Incubation of anti-human CYP2C8 IgG with human liver microsomes at a ratio of 40 µg IgG/mg microsomal protein (150 µg IgG/nmol microsomal P450) before reaction initiation will typically give 80-90% inhibition of an exemplary CYP2C8-catalyzed reaction (e.g., taxol 6α-hydroxylation; **see attached**). Methodology for conducting P450 immunoinhibition assays is given in the Protocols section.

****Anti-CYP2C8 is covered under U.S. Patent No. 6,623,960**

SPECIFIC INHIBITION OF TAXOL 6α-HYDROXYLATION IN HUMAN LIVER MICROSOMES BY ANTI-CYP2C8



Panel A - Monoclonal antibody to human CYP2C8 gave marked inhibition (92% at 40 µg IgG/mg mx protein) of taxol 6α-hydroxylase activity by human liver microsomes whereas the other P450 antibodies tested had minor effects on this CYP2C8-catalyzed activity. **Panel B** – In a separate experiment, maximal inhibition (92%) of microsomal taxol 6α-hydroxylation by the same liver microsomes was achieved at an anti-CYP2C8 IgG:P450 ratio of 40 µg IgG/mg protein. Control rates (+ preimmune IgG) of 6α-hydroxytaxol formation were 95 ± 10 pmol/min/mg protein (n = 3).



The figure depicts the inhibition obtained of hepatic taxol hydroxylation at the taxane-6α and C3'-phenyl positions upon preincubation with optimal amounts of anti-CYP2C8 MAb IgG and anti-CYP3A4 IgG, respectively. The results confirm that the former reaction is catalyzed by CYP2C8 while the latter reaction is mediated by CYP3A4.