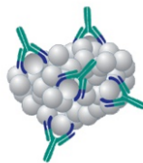


CYP450-GP



PRODUCT NUMBER Hu-A004

ANTI-CYP2C8 IgG**

Monoclonal Antibody Developed in Mice, IgG₁ Fraction
LOT 281/P1

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 lyophilization from 100 mM potassium phosphate buffer (pH 7.4).

◆ Specificity and Purity

Immunospecificity has been determined 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 IgG₁.

	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 Lyophilized Product and Storage

Store lyophilized 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

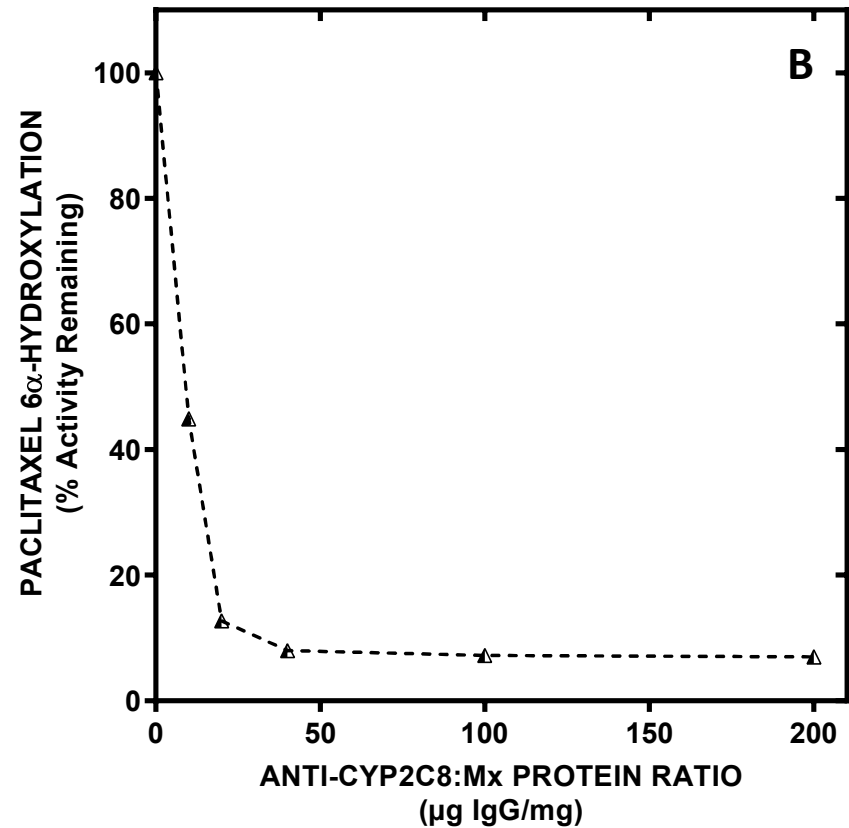
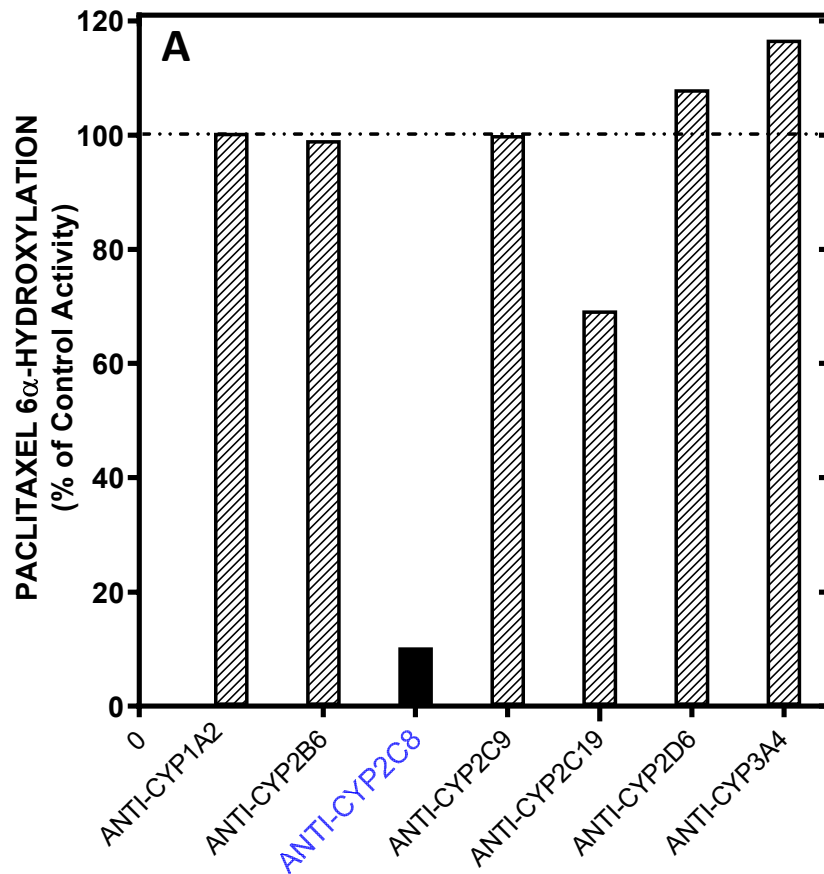
Anti-human CYP2C8 MAb IgG/ml does not react with its corresponding antigen (native or recombinant) on protein blots, and is therefore not suitable for Western blot analysis.

◆ Use for Immunoinhibition

Incubation of anti-human CYP2C8 IgG with human liver microsomes at a ratio of 150 µg IgG/nmol microsomal P450 (0.05 mg IgG/mg microsomal protein) before reaction initiation will typically give 80-90% inhibition of an exemplary CYP2C8-catalyzed reaction (e.g., paclitaxel 6 α -hydroxylation; see attached). Methodology for conducting P450 immunoinhibition assays is given in the [PROTOCOLS](#) section.

****Crude ascites fluid containing anti-CYP2C8 mAb was supplied by NCI/NIH (NIH reference #E-077-1999/0).**

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



Panel A – A monoclonal antibody to human CYP2C8 gave marked inhibition (92% at 50 μ g IgG/mg mx protein) of paclitaxel 6 α -hydroxylase activity by human liver microsomes whereas the other P450 antibodies tested had negligible effects on this CYP2C8-catalyzed activity. **Panel B** – In a separate experiment, maximal inhibition (92%) of microsomal paclitaxel 6 α -hydroxylation by the same liver microsomes was achieved at an anti-CYP2C8 IgG:P450 ratio of 40 μ g IgG/mg protein. Control rates (+ preimmune IgG) of 6 α -hydroxytaxol formation were 95 \pm 10 pmol/min/mg protein (n = 3).