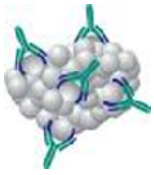
CYP450-GP



PRODUCT NUMBER Hu-A010M

ANTI-CYP1A2 IgG**

Monoclonal Antibody Developed in Mice, IgG Fraction

LOT 26.7.5

Ascites fluid containing CYP1A2 monoclonal antibodies (MAb) was produced in mice upon injection with hybridomas derived from animals immunized with recombinant human CYP1A2. The IgG fraction from a single clone was purified from ascites fluid using caprylic acid/ammonium sulfate fractionation. Anti-human CYP1A2 IgG is provided as a powder after lyophylization from 100 mM potassium phosphate buffer (pH 7.4).

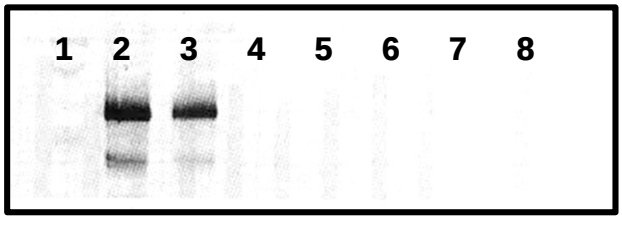
♦ Specificity and Purity

Immunospecificity has been determined by ELISA and Western blotting. As shown below, anti-human CYP1A2 MAb reacts only with its corresponding immunogen in both analyses; cross-reactivity with other human P450s is negligible. Reactivity of the MAb with the homologous CYP1A 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-CYP1A2	**+++**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

ELISA absorbance values ≥ 1.0 are designated as **+++** whereas values ≤ 0.15 are denoted as 0.



Anti-CYP1A2 mAb Reactivity with Expressed P450s▲

Lane 1 = Blank
Lane 2 = CYP1A2 Supersomes (0.1 µg)
Lane 3 = CYP1A2 Hepa G2 cells (0.1 µg)
Lane 4 = CYP1A1 Supersomes (1 µg)
Lane 5 = CYP2A6 Supersomes (1 µg)
Lane 6 = CYP2C9 Supersomes (1 µg)
Lane 7 = CYP2E1 Supersomes (1 µg)
Lane 8 = CYP3A4 Supersomes (1 µg)

♦ 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

Incubate blots overnight with 20-40 µg mouse anti-human CYP1A2 MAb IgG/ml of blocking solution. After washing to remove unbound CYP1A2 antibody, incubate with an anti-mouse IgG conjugate of choice (e.g, anti-mouse IgG-peroxidase), and develop accordingly. A detailed Western blotting method is given in the PROTOCOLS section.

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♦ Use for Immunoinhibition

Incubation of anti-human CYP1A2 IgG with human liver microsomes at a ratio of **80 µg IgG**/mg microsomal protein before reaction initiation will typically give 80-85% inhibition of an exemplary CYP1A2-catalyzed reaction (e.g., phenacetin *O*-deethylation; **see below**). The methodology for conducting P450 immunoinhibition assays is given in the PROTOCOLS section.

******Crude ascites fluid containing anti-CYP1A2 mAb was supplied by NCI/NIH (NIH reference #E-077-1999/0).
▲Taken from: Yang TJ et al: *Pharmacogenetics* 8:375-382, 1998

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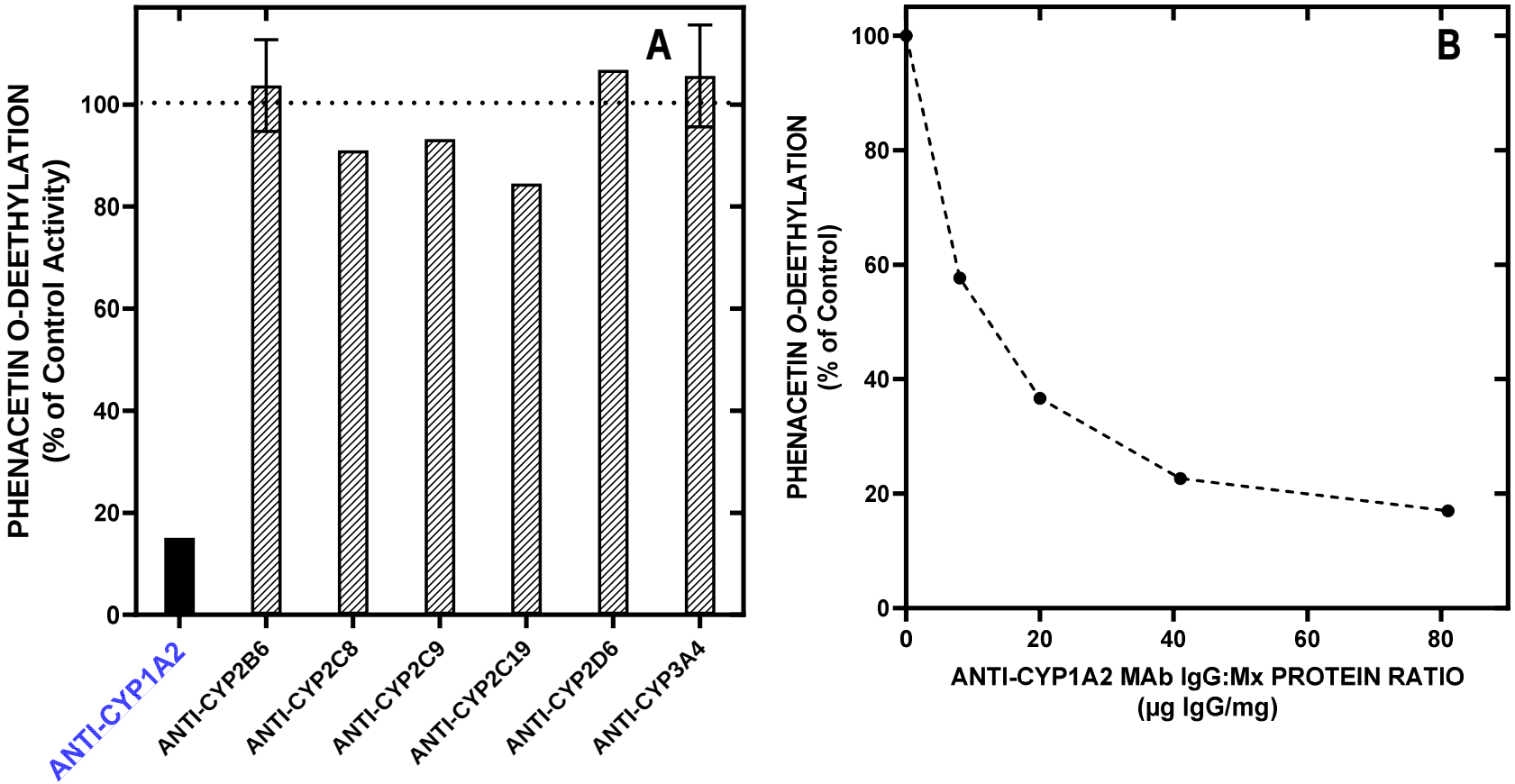
SPECIFIC INHIBITION OF PHENACETIN *O*-DEETHYLATION IN HUMAN LIVER MICROSOMES BY CYP1A2 mAb



Panel A – Monoclonal antibodies to human CYP1A2 had a marked inhibitory effect (85% inhibition at 40 µg IgG/mg microsomal protein) on phenacetin *O*-deethylation by human liver microsomes whereas the other P450 antibodies tested had neglible effects on this CYP1A2-catalyzed reaction. Values shown denote the average of duplicate determinations or the mean ± SD of triplicate determinations.

Panel B – Immunotitration experiments revealed that maximal inhibition (83%) of phenacetin *O*-deethylation was achieved at an anti-CYP1A2 mAb:Mx protein ratio of 80 µg/mg. Rates of phenacetin metabolism (in the presence of control IgG) ranged from 0.73 – 0.81 nmol APAP formed/min/mg protein.