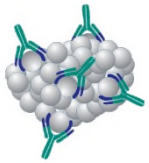
CYP450-GP



PRODUCT NUMBER Hu-A012

ANTI-HUMAN CYP2B6 IgG**

Monoclonal Antibody Developed in Mice, IgG Fraction

LOT 49.10.20

Ascites fluid containing CYP2B6 monoclonal antibodies (MAb) was produced in mice upon injection with hybridomas derived from animals immunized with recombinant human CYP2B6. The whole IgG fraction was purified from ascites fluid using caprylic acid/ammonium sulfate fractionation. Anti-human CYP2B6 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 and Western blotting. As shown below, anti-human CYP2B6 MAb IgG reacts only with its corresponding immunogen in human liver microsomes. Cross-reactivity with other P450s is negligible. Reactivity of the monoclonal antibody with the homologous CYP2B 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-CYP2B6	**0**	**+++**	**0**	**0**	**0**	**0**	**0**	**0**

♦ 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 CYP2B6 MAb IgG/ml of blocking solution. After washing to remove unbound CYP2B6 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.

♦ Use for Immunoinhibition

Incubation of anti-human CYP2B6 IgG with human liver microsomes at a ratio of **80 µg IgG**/mg microsomal protein (150 µg IgG/nmol microsomal P450) before reaction initiation will typically give 80-90% inhibition of an exemplary CYP2B6-catalyzed reaction (e.g., buproprion hydroxylation; **see attached**). Methodology for conducting P450 immunoinhibition assays is given in the Protocols section.

****Anti-CYP2B6 is covered under U.S. Patent No. 6,623,960**

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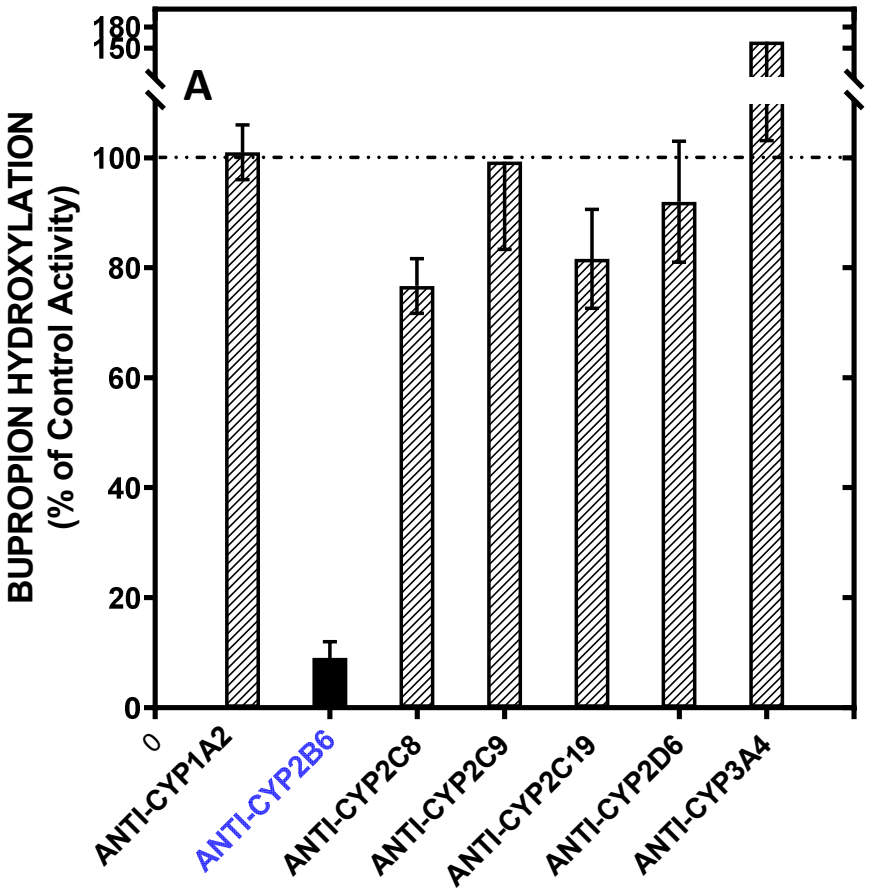
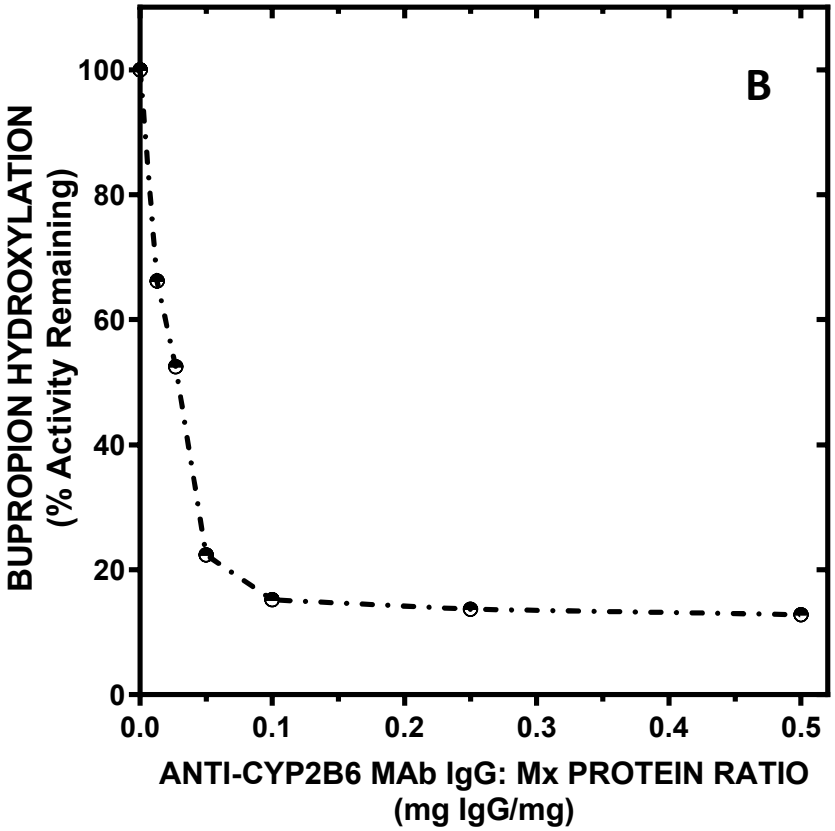
SPECIFIC INHIBITION OF BUPROPION HYDROXYLATION IN HUMAN LIVER MICROSOMES BY ANTI-CYP2B6





Panel A - Antibodies to human CYP2B6 elicited potent inhibition (91% at 0.25 mg IgG/mg protein) of bupropion hydroxylation by human liver microsomes. The other antibodies examined gave either negligible or modest but nonspecific inhibition of microsomal bupropion oxidation to hydroxybupropion; anti-human CYP3A4 stimulated this reaction. **Panel B** - In a separate experiment, maximum inhibition (86%) of bupropion metabolism was achieved using an anti-CYP2B6 IgG:microsomal protein ratio of 0.2 mg/mg. Control (+ preimmune IgG) rates of bupropion hydroxylation were 150 pmol hydroxybupropion formed/min/mg protein.