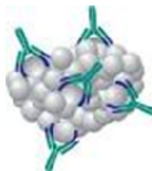
CYP450-GP



PRODUCT Hu-A009

ANTI-HUMAN CYP2D6 IgG

Polyclonal Antibody Developed in Rabbits, IgG Fraction

LOT Ra5318/B#3-5

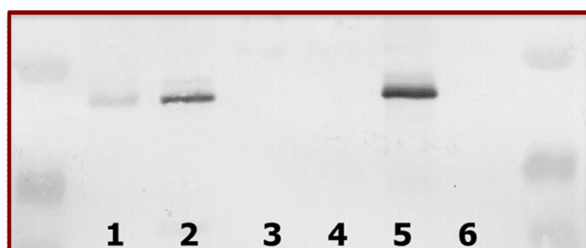
Antiserum was developed in rabbits using recombinant human CYP2D6 as immunogen. The whole IgG fraction was purified from antiserum using caprylic acid/ammonium sulfate fractionation. Anti-human CYP2D6 IgG is provided as a powder after lyophylization from 100 mM potassium phosphate buffer (pH 7.4), 150 mM KCl, and 2.5 µM thimerosal (added as a preservative). .

♦ Specificity and Purity

Specificity has been determined by Western blotting. Anti-human CYP2D6 IgG reacts with only its corresponding 49 kDa immunogen in human liver microsomes. Cross-reactivity with homologous CYP2D proteins in rat and mouse liver microsomes has not been determined. Antibody purity has been established by SDS-PAGE, where two protein bands with molecular weights of 50 kDa and 25 kDa, corresponding to the heavy and light chains, respectively, of rabbit IgG, can be visualized by Coomassie blue staining.

♦ Reconstitution of Lyophylized Product and Storage

Store lyophylized product at 0-5°C. For Western blotting, reconstitute to 1 mg IgG protein/ml final concentration by adding the appropriate amount of PBS/50% glycerol to the vial of lyophylized IgG and mixing gently until powder dissolves. The solution can then be stored at -20°C, as the presence of 50% glycerol will prevent freeze/thawing. For immunoinhibition studies, reconstitute anti-CYP2D6 IgG in an appropriate buffer (e.g., 100 mM potassium phosphate, pH 7.4) to a concentration of 10-20 mg IgG/ml, and also store at -20°C. In the absence of glycerol, however, the number of freeze/thaw cycles should be kept to a minimum.



Anti-CYP2D6 IgG Reactivity with Human Liver Proteins
Lane 1 = Liver Microsomes from Subject A (15 µg)
Lane 2 = Purified CYP2D6 (0.1 µg)
Lane 3 = Purified CYP3A4 (0.1 µg)
Lane 4 = Purified CYP2C9 (0.1 µg)
Lane 5 = CYP2D6 Supersomes (1 µg)
Lane 6 = Liver microsomes from Subject B (15 µg)
[Subject A and B have the EM and PM phenotypes, respectively].

♦ Use for Western Blotting

Incubate blots overnight with 2.5 - 5.0 µg rabbit anti-human CYP2D6 IgG/ml of blocking solution. After washing to remove unbound CYP2D6 antibody, incubate with an anti-rabbit IgG conjugate of choice (e.g, anti-rabbit IgG-peroxidase), and develop accordingly. A detailed Western blotting method is given in the Protocols section.

♦ Use for Immunoinhibition

Incubation of anti-human CYP2D6 IgG with human liver microsomes at a ratio of 2 mg IgG/mg microsomal protein (7.5 mg IgG/nmol microsomal P450) before reaction initiation will typically give 80% inhibition of an exemplary CYP2D6-catalyzed reaction (e.g., dextromethorphan *O*-demethylation). Methodology for conducting P450 immunoinhibition assays is given in the Protocols section.

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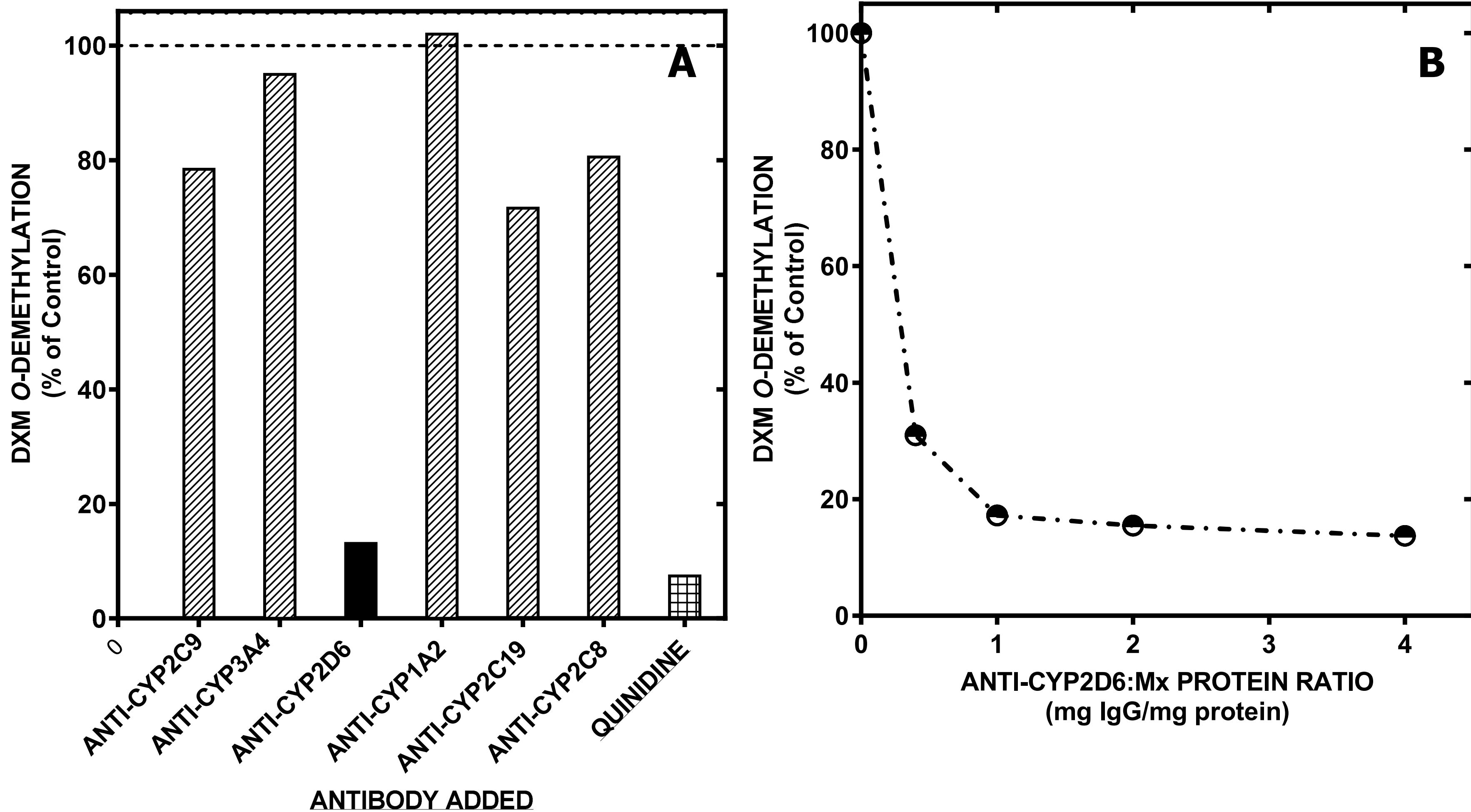

Panel A - Antibodies to human CYP2D6 elicited potent inhibition (87% at 2 mg IgG/mg protein) of DXM *O*-demethylation by liver microsomes (pool of 50 subjects). The other antibodies examined gave either negligible or modest but <u>nonspecific</u> inhibition (< 25%) of DXM oxidation to DXO. The effect obtained with the CYP2D6 chemical inhibitor quinidine (4 μM) is shown for comparison. **Panel B** - In a separate experiment, maximum inhibition (87%) of DXM metabolism was achieved using an anti-CYP2D6 IgG:microsomal protein ratio of 3.5 mg/mg. Control (+ preimmune IgG) rates of DXM *O*-demethylation were 0.23 nmol dextrorphan formed/min/mg protein.