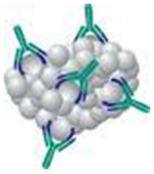
CYP450-GP



PRODUCT NUMBER Hu-A002

ANTI-CYP2E1 IgG

Polyclonal Antibody Developed in Rabbits, IgG Fraction

LOT RaBB/B#2-7

Antiserum was developed in rabbits using purified recombinant human CYP2E1 as immunogen. The whole IgG fraction was purified from antiserum using caprylic acid/ammonium sulfate fractionation. Anti-human CYP2E1 IgG is provided either 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) or as a solution in the same buffer.

♦ Specificity and Purity

Specificity has been determined by Western blotting (see below). Anti-human CYP2E1 IgG reacts with only its corresponding 55 kDa immunogen in human liver microsomes. Antibody purity has been established by SDS-PAGE run under denaturing conditions which, upon Coomassie blue staining, gives two protein bands with molecular weights of 50 kDa and 25 kDa corresponding to the heavy and light chains, respectively, of rabbit IgG.

♦ Reconstitution of Lyophylized Product and Storage

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

Immunoreactivity of anti-CYP2E1 IgG with human liver proteins.

Lane A = Liver microsomes from Subject A (15 µg)
Lane B = Purified CYP2E1 (0.1 µg)
Lane C = Purified CYP4A11 (0.1 µg)
Lane C = Liver microsomes from Subject B (15 µg)

♦ Use for Western Blotting

Incubate blots overnight with 2.5 - 5.0 µg rabbit anti-human CYP2E1 IgG/ml of appropriate blocking solution. After washing to remove unbound CYP2E1 antibody, incubate with an anti-rabbit IgG conjugate of choice (e.g, anti-rabbit IgG-peroxidase or anti-rabbit IgG-biotin), and develop accordingly. A detailed Western blotting method can be found in the PROTOCOLS section.

♦ Use for Immunoinhibition

Pre-incubation of anti-human CYP2E1 IgG with human liver microsomes at a ratio of 2.0 mg IgG/mg microsomal protein (i.e., 5 mg IgG/nmol microsomal P450) before reaction initiation will typically give 80-90% inhibition of an exemplary CYP2E1-catalyzed reaction (e.g., chlorzoxazone 6-hydroxylation). Methodology for conducting P450 immunoinhibition assays is given in the PROTOCOLS section.

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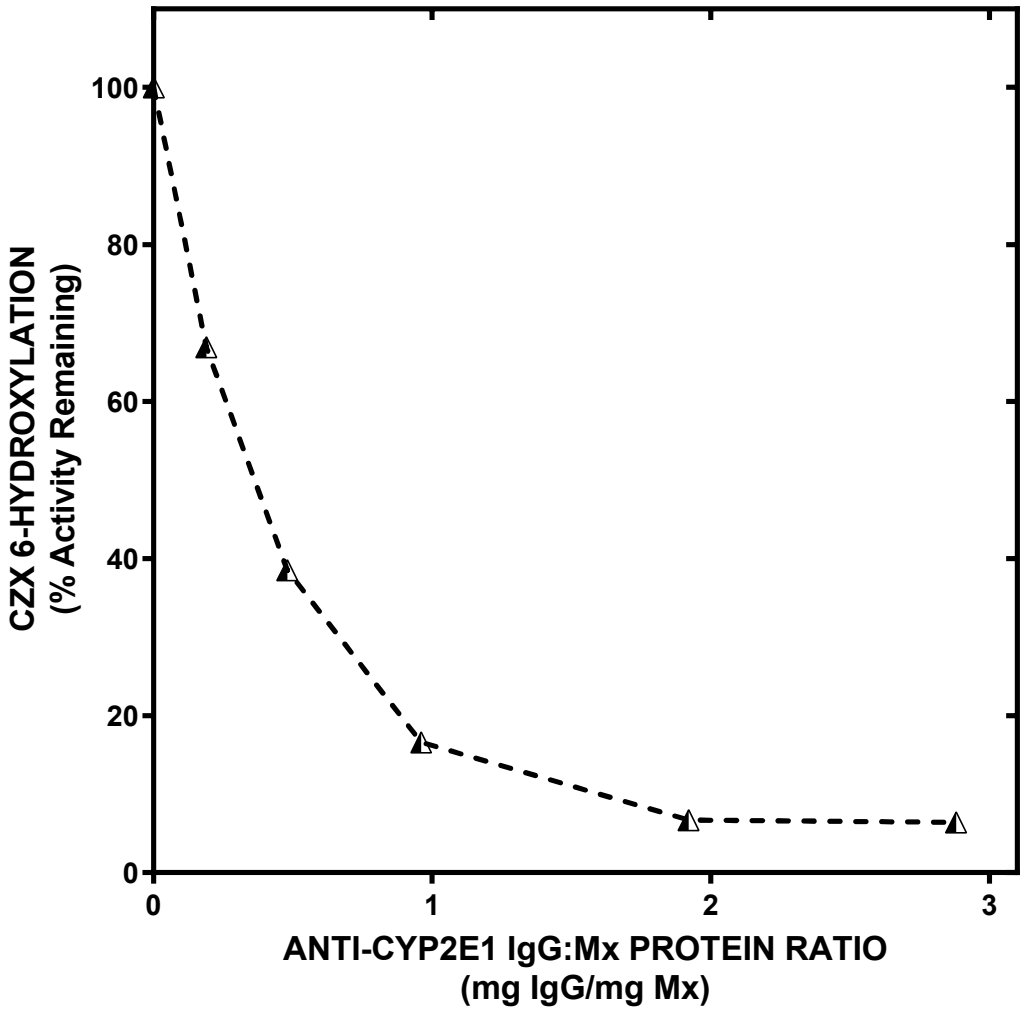
SPECIFIC INHIBITION OF CHLORZOXAZONE 6-HYDROXYLATION IN HUMAN LIVER MICROSOMES BY ANTI-CYP2E1



Maximal inhibition (93%) of chlorzoxazone 6-hydroxylase activity by liver microsomes from subject UC8905 was achieved using an anti-CYP2E1 IgG:mx protein ratio of 1.9 mg/mg. Control rates (+ preimmune IgG) of chlorzoxazone metabolism were 3.36 nmol 6-hydroxychlorzoxazone formed/min/mg protein. In experiments not shown, antibodies to CYP3A4 or CYP2C9 had negligible effects on the conversion of chlorzoxazone to its 6-hydroxylated metabolite.