CYP450-GP

PRODUCT NUMBER Hu-A010

ANTI-CYP1A2 IgG

Polyclonal Antibody Developed in Rabbits, IgG Fraction

LOT Ra8013A B#3-5

Antiserum was developed in rabbits using purified recombinant human CYP1A2 as immunogen. The whole IgG fraction was purified from antiserum using caprylic acid/ammonium sulfate fractionation. Anti-CYP1A2 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).

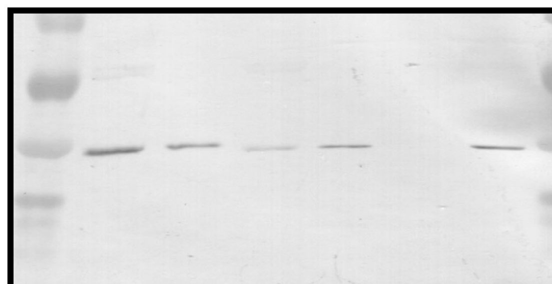
♦ Specificity and Purity

Specificity has been determined by Western blotting. Anti-CYP1A2 IgG reacts with both CYP1A2 and CYP1A1 (see below), the former of which is found in human liver while the latter is expressed in extrahepatic tissues only.

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 rabbit IgG.

♦ Reconstitution of Lyophylized Product and Storage

Store lyophylized product at 0-5°C. For Western blotting, the IgG should be reconstituted to 1 mg 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. Afterwards, the solution can be stored at -20°C, as the presence of 50% glycerol will prevent freeze/thawing. For immunoinhibition studies, reconstitute anti-CYP1A2 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.



1 2 3 4 5 6

Anti-CYP1A2 IgG Immunoreactivity with Liver Proteins

1 = Subject A Liver Mx	20 µg
2 = rCYP1A2 Supersomes	0.5 µg
3 = Subject B Liver Mx	20 µg
4 = rCYP1A1 (in Sf9 insect cells)	5 µg
5 = Purified Human CYP3A4	0.1 µg
6 = Subject C Liver Mx	20 µg

♦ Use for Western Blotting

Incubate blots overnight with 2.5-5.0 µg rabbit anti-human CYP1A2 IgG/ml of appropriate blocking solution. After washing to remove unbound CYP1A2 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

Incubation of anti-human CYP1A2 IgG with human liver microsomes at a ratio of 3.0 mg IgG/mg mx protein (7.5 mg IgG/nmol microsomal P450) before reaction initiation will typically give 80% inhibition of an exemplary CYP1A2-catalyzed reaction (e.g., phenacetin *O*-deethylation; see attached). The methodology for conducting P450 immunoinhibition assays is given in the PROTOCOLS section.

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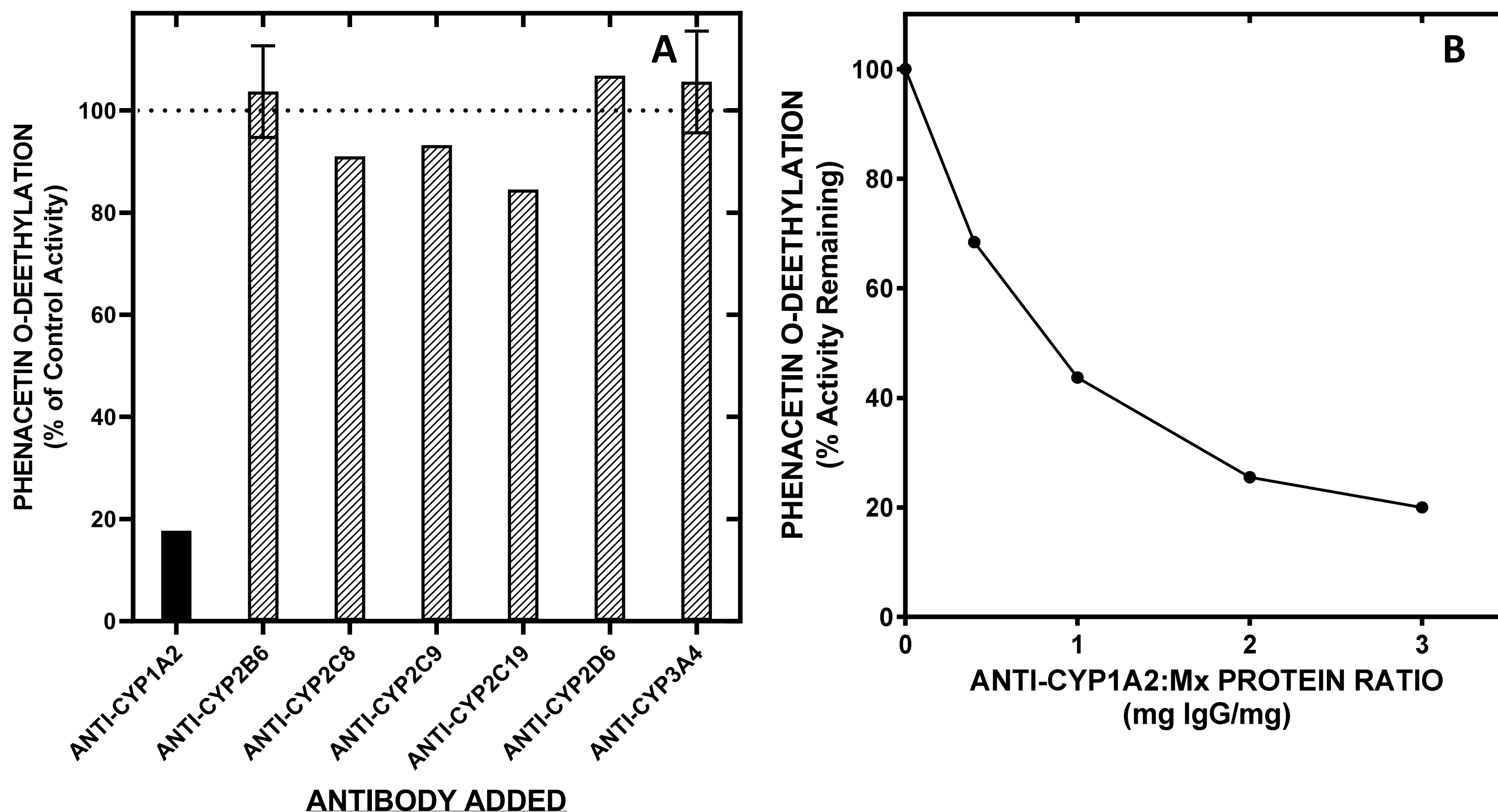

Panel A - Antibodies to human CYP1A2 had a marked inhibitory effect (82% inhibition at 3.0 mg IgG/mg microsomal protein) on phenacetin *O*-deethylation by human liver microsomes whereas the other P450 antibodies tested had neglible effects on this CYP1A2-catalyzed reaction. Values shown denote the average of duplicate determinations or the mean ± SD of triplicate determinations.
Panel B – An immunotitration experiment revealed that maximal inhibition (80%) of phenacetin *O*-deethylation was achieved at an anti-CYP1A2 IgG:mx protein ratio of 3.0 mg/mg.