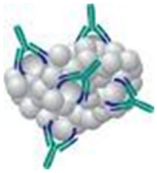
CYP450-GP



PRODUCT NUMBERS Hu-A011A, Hu-A011B

CYP ImmunoInhibit Kit

Inhibitory Antibodies to CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 & CYP3A4✺
Developed in Rabbits and Mice, IgG Fraction
LOT 2020a

Antisera were developed in rabbits using purified human native or recombinant CYP1A2, CYP2C19, CYP2D6 and CYP3A4 as immunogens. Ascites fluid was produced in mice using hybridomas derived from animals immunized with recombinant human CYP2B6, CYP2C8 and CYP2C9. Whole IgG fractions were purified from antisera or ascites fluid using caprylic acid/ammonium sulfate fractionation. Preimmune (control) IgG was derived from rabbit serum prior to immunization. The individual polyclonal IgGs (1-2 mg) and mAb IgGs (0.1-0.2 mg) are provided as powders after lyophylization from 100 mM potassium phosphate buffer (pH 7.4) containing 150 mM KCl.

♦ Specificity and Purity

Antibody specificity, as determined by Western blotting and/or ELISA (http://cyp450-gp.com/about/p450-antibodies), is summarized below.

	P450 ENZYME								
ANTIBODY	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4	CYP3A5	CYP4A11
Anti-CYP1A2	**+++**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Anti-CYP2B6	**0**	**+++**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Anti-CYP2C8	**0**	**0**	**+++**	**0**	**0**	**0**	**0**	**0**	**0**
Anti-CYP2C9	**0**	**0**	**0**	**+++**	**0**	**0**	**0**	**0**	**0**
Anti-CYP2C19	**0**	**0**	**0**	**0**	**+++**	**0**	**0**	**0**	**0**
Anti-CYP2D6	**0**	**0**	**0**	**0**	**0**	**+++**	**0**	**0**	**0**
Anti-CYP3A4	**0**	**0**	**0**	**0**	**0**	**0**	**+++**	**++**	**0**

Antibody purity has been established by SDS-PAGE run under denaturing conditions. In each case, 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 and/or mouse IgG.

♦ Reconstitution of Lyophylized Product and Storage

Store kit containing the lyophylized IgGs at -20°C. Reconstitute by adding 100-200 µl of 100 mM potassium phosphate buffer, pH 7.4 (or another suitable buffer) to each vial of polyclonal IgG, and mix vial gently until powder dissolves, giving a final concentration of 10 mg IgG/ml. CYP2B6, CYP2C8 and CYP2C9 mAb IgGs are reconstituted in the same manner but the final concentration will be 1.0 mg IgG/ml. After reconstitution, the antibody solutions should be stored at -20°C, taking care to avoid extensive freeze/thaw cycles.

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♦ Use for Immunoinhibition with Human Liver Microsomes✷

Antibody	Substrate/Reaction	Extent of Inhibition
Anti-CYP1A2	Phenacetin *O*-Deethylation	> 80% at 3.0 mg IgG/mg microsomal protein
Anti-CYP2B6	**Bupropion Hydroxylation**	**>85% at 0.05 mg IgG/mg microsomal protein**
Anti-CYP2C8	Paclitaxel 6α-Hydroxylation	> 85% at 0.04 mg IgG/mg microsomal protein
Anti-CYP2C9	Diclofenac 4'-Hydroxylation	> 80% at 0.075 mg IgG/mg microsomal protein
Anti-CYP2C19	S-Mephenytoin 4-Hydroxylation	> 90% at 1.0 mg IgG/mg microsomal protein
Anti-CYP2D6	Dextromethorphan *O*-Demethylation	> 80% at 2 mg IgG/mg microsomal protein
Anti-CYP3A4	Nifedipine Oxidation	> 85% at 2 mg IgG/mg microsomal protein

✷Typical inhibition studies using the antibodies contained in the CYP ImmunoInhibit Kit are attached; the assay conditions employed are given in the specification sheets for the individual antibodies that comprise the kit. Deployment of the kit itself is described in the **MANUAL** that accompanies this product.

♦ Use for Western Blotting

Incubate blots overnight with 5-10 µg rabbit anti-human CYPxxx IgG/ml of appropriate blocking solution. Use 0.5-1.0 µg mouse anti-human CYP2B6 mAb IgG/ml of blocking solution. [Anti-human CYP2C8 and CYP2C9 are both mAb that do not react with their cognate antigens on protein blots, and are thus not suitable for Western blot analysis]. After washing to remove unbound primary antibody, incubate with an anti-rabbit or anti-mouse IgG conjugate of choice (e.g, anti-IgG-peroxidase or anti-IgG-biotin), and develop accordingly. A detailed Western blotting method can be found in the **PROTOCOLS** section.

****Anti-human CYP2B6, anti-human CYP2C8 and anti-human CYP2C9 are covered under U.S. Patent No. 6,623,960.**

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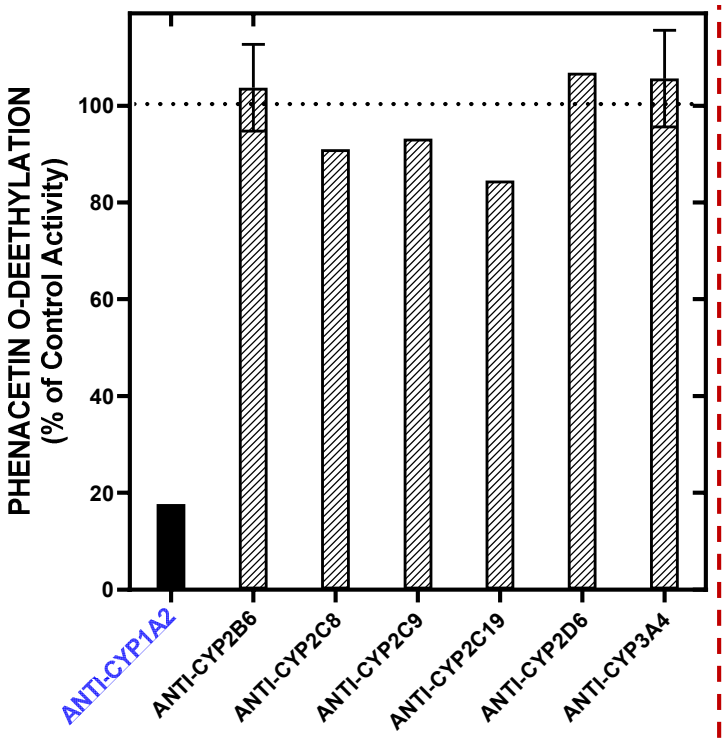
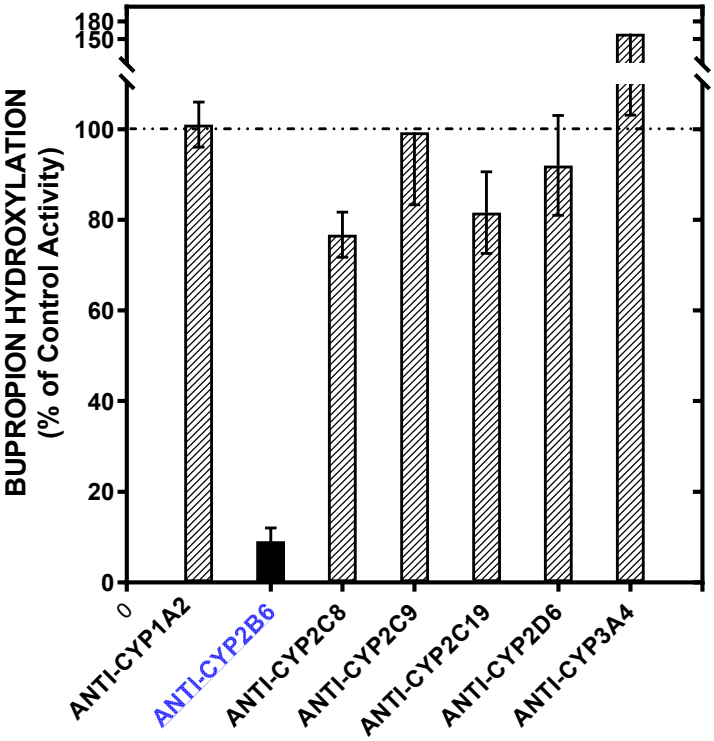
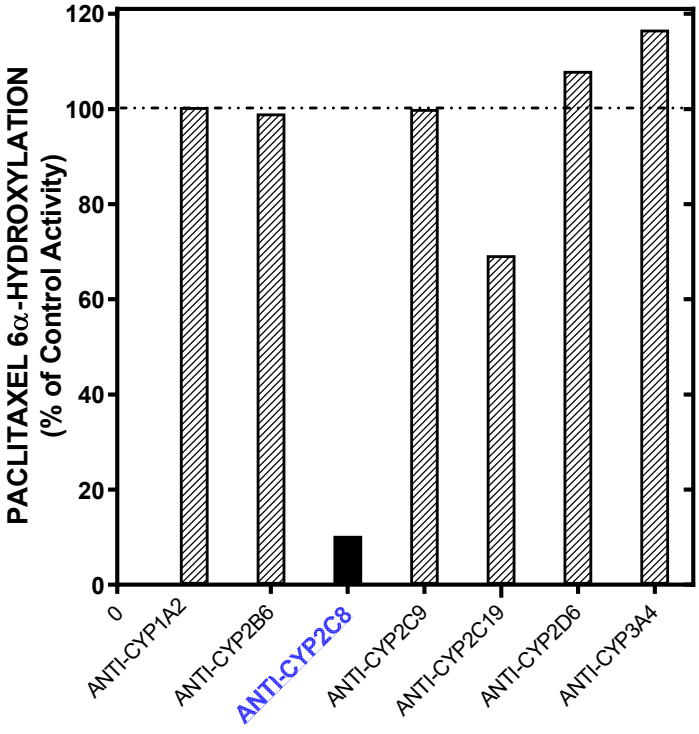
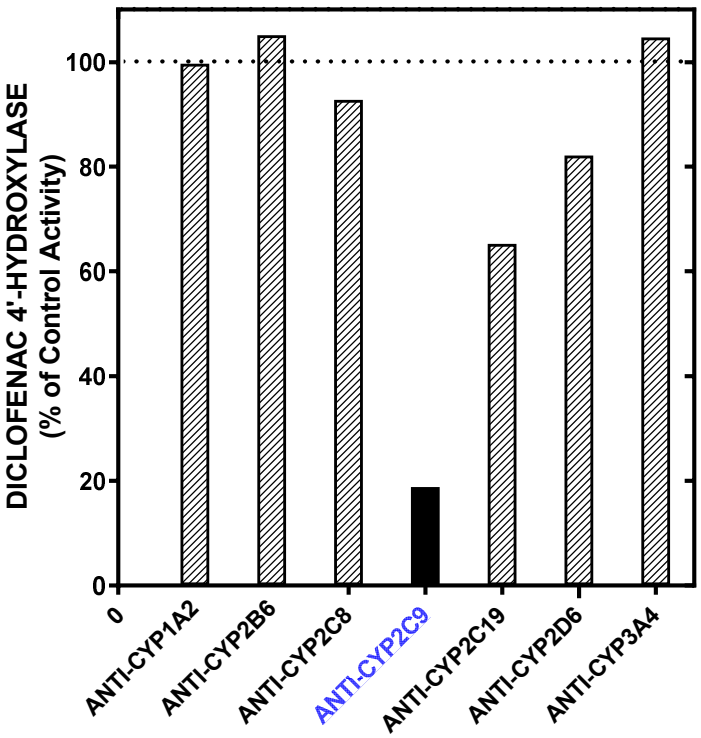
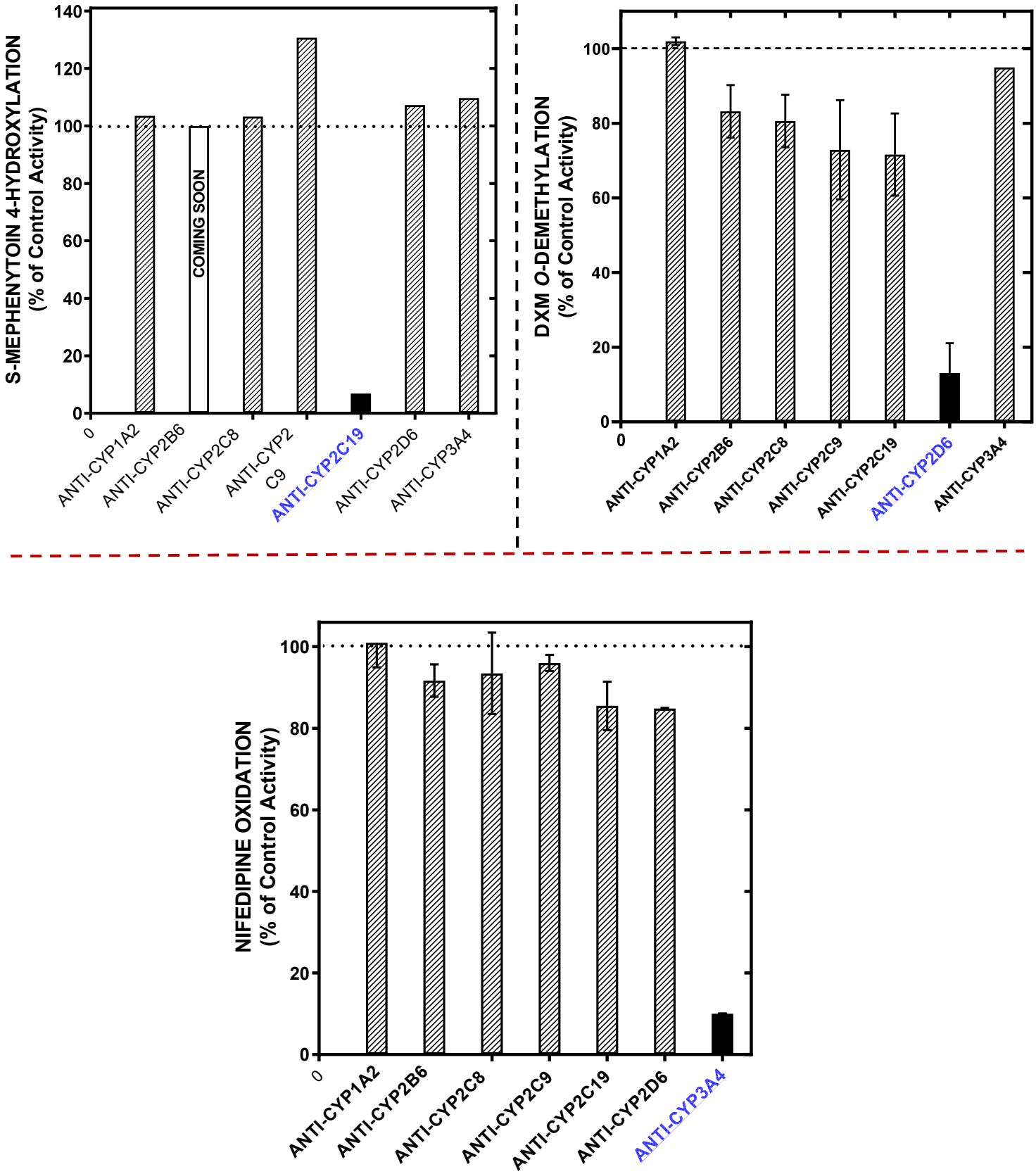
SPECIFIC INHIBITION OF MICROSOMAL DRUG OXIDATION BY *CYP IMMUNOINHIBIT KIT* ANTIBODIES



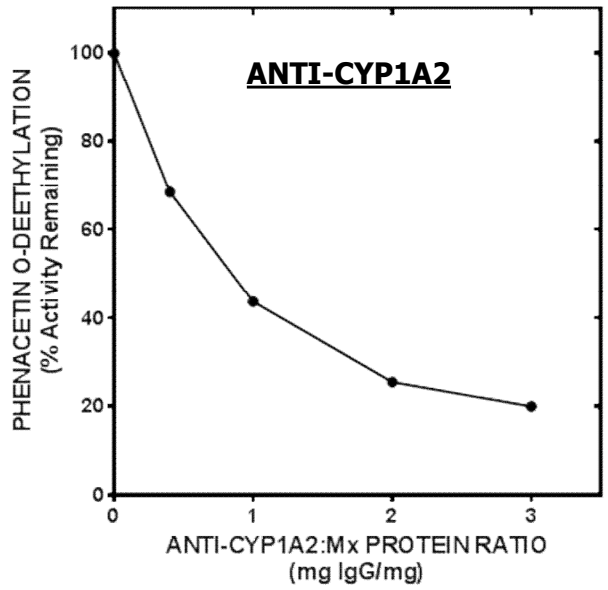


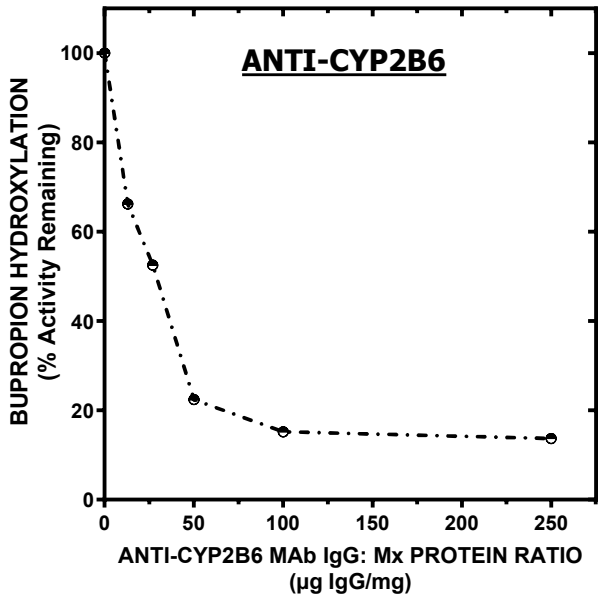


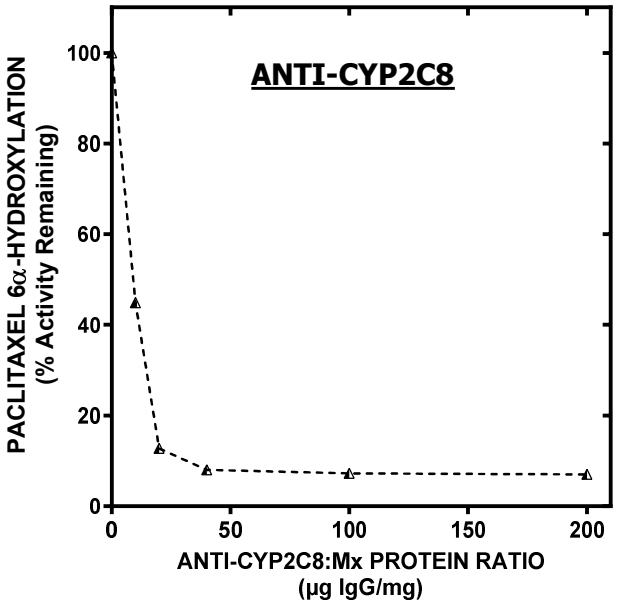


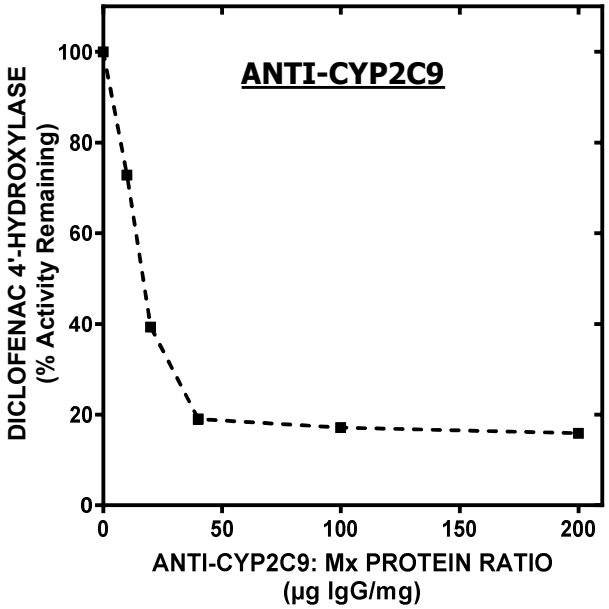

S-MEPHENYTOIN 4-HYDROXYLATION
(% of Control Activity)
140
120
100
80
60
40
20
0
COMING SOON
0
ANTI-CYP1A2
ANTI-CYP2B6
ANTI-CYP2C8
ANTI-CYP2C9
ANTI-CYP2C19
ANTI-CYP2D6
ANTI-CYP3A4
DXM O-DEMETHYLATION
(% of Control Activity)
100
80
60
40
20
0
0
ANTI-CYP1A2
ANTI-CYP2B6
ANTI-CYP2C8
ANTI-CYP2C9
ANTI-CYP2C19
ANTI-CYP2D6
ANTI-CYP3A4
NIFEDIPINE OXIDATION
(% of Control Activity)
100
80
60
40
20
0
0
ANTI-CYP1A2
ANTI-CYP2B6
ANTI-CYP2C8
ANTI-CYP2C9
ANTI-CYP2C19
ANTI-CYP2D6
ANTI-CYP3A4

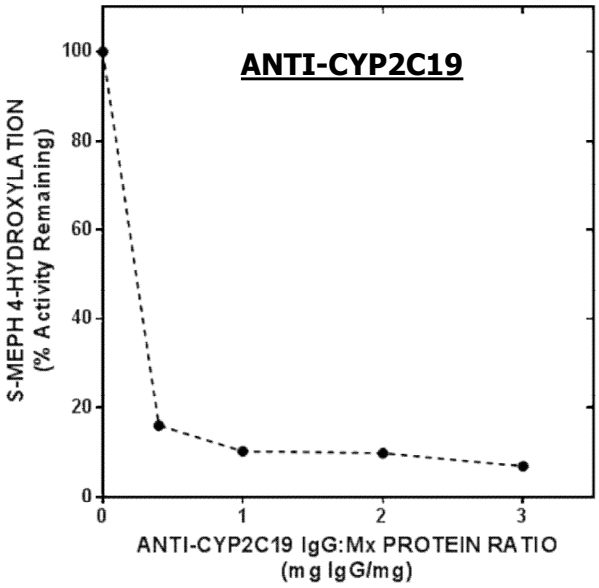
DOSE-RESPONSE INHIBITION OF MICROSOMAL DRUG OXIDATION BY *CYP IMMUNOINHIBIT KIT* ANTIBODIES

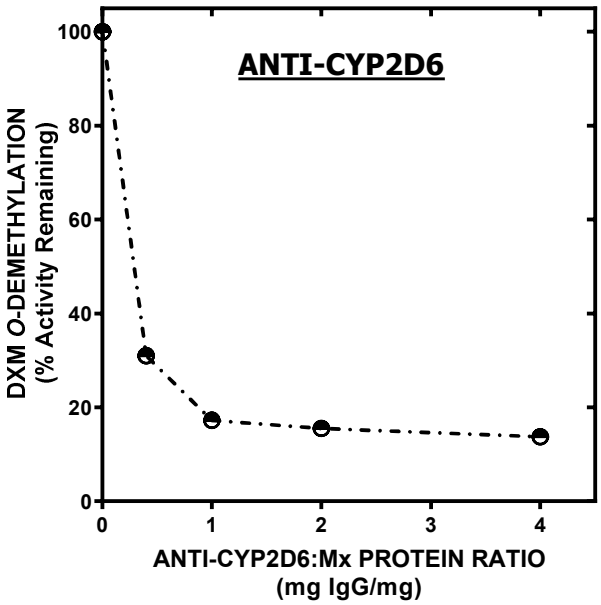


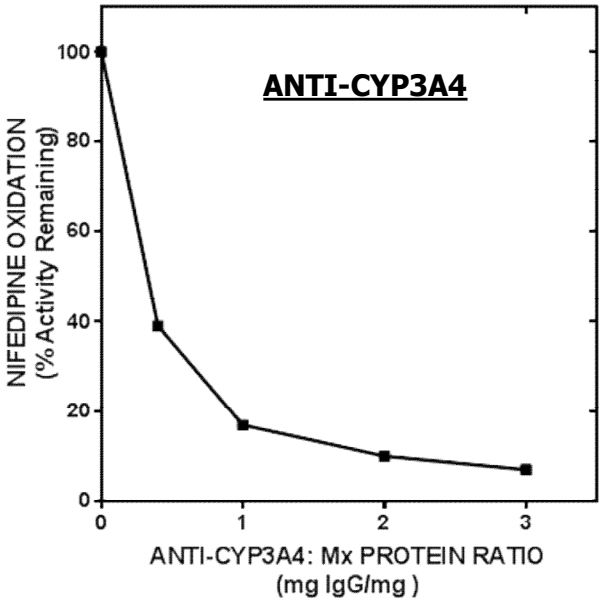







ANTI-CYP2C19
S-MEPH 4-HYDROXYLATION
(% Activity Remaining)
100
80
60
40
20
0
0
1
2
3
ANTI-CYP2C19 IgG:Mx PROTEIN RATIO
(mg IgG/mg)


ANTI-CYP2D6
DXM O-DEMETHYLATION
(% Activity Remaining)
100
80
60
40
20
0
0
1
2
3
4
ANTI-CYP2D6:Mx PROTEIN RATIO
(mg IgG/mg)


ANTI-CYP3A4
NIFEDIPINE OXIDATION
(% Activity Remaining)
100
80
60
40
20
0
0
1
2
3
ANTI-CYP3A4: Mx PROTEIN RATIO
(mg IgG/mg)