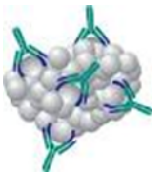
CYP450-GP



PRODUCT NUMBERS Hu-A011A, Hu-A011B

CYP ImmunoInhibit Kit

Inhibitory Antibodies to CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 & CYP3A4
Developed in Rabbits and Mice, IgG Fraction
LOT 2014a

Antisera were developed in rabbits using purified human native or recombinant CYP1A2, CYP2C9, CYP2C19, CYP2D6 or CYP3A4 as immunogens. Ascites fluid was produced in mice using hybridomas derived from animals immunized with recombinant human CYP2C8. 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 IgG (0.075-0.15 mg) are provided as powders 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

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

	P450 ENZYME							
ANTIBODY	**CYP1A2**	**CYP2A6**	**CYP2C8**	**CYP2C9**	**CYP2C19**	**CYP2D6**	**CYP3A4**	**CYP3A5**
Anti-CYP1A2	**+++**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Anti-CYP2C8	**0**	**0**	**+++**	**0**	**0**	**0**	**0**	**0**
Anti-CYP2C9	**0**	**0**	**+/-**	**+++**	**+**	**0**	**0**	**0**
Anti-CYP2C19	**0**	**0**	**+/-**	**+**	**+++**	**0**	**0**	**0**
Anti-CYP2D6	**0**	**0**	**0**	**0**	**0**	**+++**	**0**	**0**
Anti-CYP3A4	**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 0-5°C. Reconstitute by adding 100-200 µl of100 mM potassium phosphate buffer, pH 7.4 (or another suitable reaction buffer) toeach vial of polyclonal IgG, and mix vial gently until powder dissolves, giving a final concentration of 10 mg IgG/ml. CYP2C8 MAb IgG is reconstituted in the same manner but the final concentration will be 0.75 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-CYP2C19	Omeprazole 5'-Hydroxylation	> 85% at 5 mg IgG/nmol microsomal P450
Anti-CYP2D6	Dextromethorphan *O*-Demethylation	> 80% at 10 mg IgG/nmol microsomal P450
Anti-CYP2C8	Paclitaxel 6α-Hydroxylation	> 85% at 0.15 mg IgG/nmol microsomal P450
Anti-CYP2C9	Tolbutamide Methyl Hydroxylation	> 85% at 5 mg IgG/nmol microsomal P450
Anti-CYP3A4	Nifedipine Oxidation	> 85% at 5 mg IgG/nmol P450
Anti-CYP1A2	Phenacetin *O*-Deethylation	> 85% at 5 mg IgG/nmol microsomal P450

Inhibition studies using the antibodies contained in the CYP ImmunoInhibit kit are described in detail 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. In the case of CYP2C8 mAb, 0.5 - 1.0 µg IgG/ml blocking solution is adequate. Anti-human CYP2C8 MAb does not react with its cognate antigen (native or recombinant) on protein blots, and is therefore not suitable for Western blot analysis. After washing to remove unbound primary antibody, incubate with an anti-rabbit 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 CYP2C8 is covered under U.S. Patent No. 6,623,960**

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