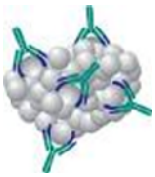
CYP450-GP



PRODUCT Hu-A001

ANTI-HUMAN CYP2A6 IgG

Polyclonal Antibody Developed in Rabbits, IgG Fraction

LOT RaG/B#6+7

Antiserum was developed in rabbits using purified human liver CYP2A6 as the immunogen. The whole IgG fraction was purified from antiserum using caprylic acid/ammonium sulfate fractionation. Anti-human CYP2A6 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).

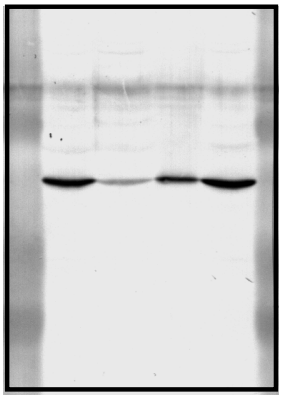
♦ Specificity and Purity

Specificity has been determined by Western blotting (see below). Anti-human CYP2A6 IgG reacts with only its corresponding 49 kDa immunogen in human liver microsomes. The antibody also recognizes homologous CYP2A proteins in rat and mouse liver microsomes. Specificity with liver homogenates or S-9 has not been determined.

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.



A B C D

Immunoreactivity of Anti-CYP2A6 IgG with human liver proteins.
Lane A = Liver microsomes from Subject A (10 µg)
Lane B = Liver microsomes from Subject B (10 µg)
Lane C = Purified CYP2A6 (0.1 µg)
Lane D = Liver microsomes from Subject C (10 µg)

♦ Use for Western Blotting

Incubate blots overnight with 2.5-5.0 µg rabbit anti-human CYP2A6 IgG/ml of appropriate blocking solution. After washing to remove unbound CYP2A6 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 complete Western blotting method can be found in the Protocols section.

♦ Use for Immunoinhibition

Incubation of anti-human CYP2A6 IgG with human liver microsomes at a ratio of 2 mg IgG/mg microsomal protein (5 mg IgG/nmol microsomal P450) before reaction initiation will typically give 95% inhibition of an exemplary CYP2A6-catalyzed reaction (e.g., coumarin 6-hydroxylation). Methodology for conducting P450 immunoinhibition assays is given in the Protocols section.

CYP450-GP, 2870 Scott Street Suite 103, Vista, CA 92081
Phone 760.295.7426; Fax 760.418.6472; sales@cyp450-gp.com