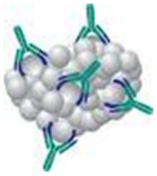
# *CYP450-GP*



**PRODUCT Hu-A006**

## ANTI-HUMAN CYP4F+ IgG

Polyclonal Antibody Developed in Rabbits, IgG Fraction

**LOT RaP/B#4+5**

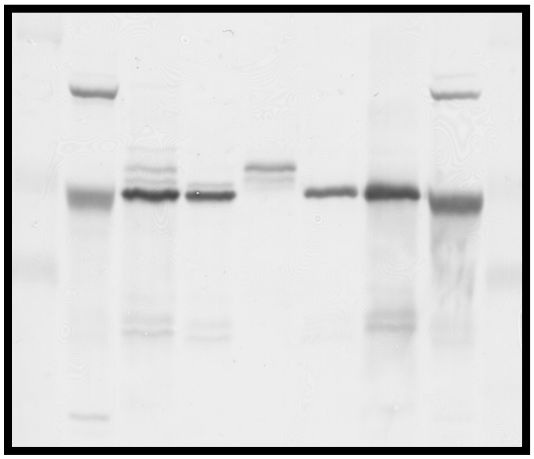
Antiserum was developed in rabbits using purified human liver CYP4F2 as immunogen. The whole IgG fraction was purified from antiserum using caprylic acid/ammonium sulfate fractionation. Anti-human CYP4F+ IgG is provided in a lyophilized state after freeze-drying from 100 mM potassium phosphate buffer (pH 7.4), 150 mM KCl, and 2.5 µM thimerosal (added as a preservative).

### ♦ Specificity and Purity

Specificity has been determined on Western blots (see below). Anti-human CYP4F+ IgG reacts strongly with CYP4F2 and several other 56-57 kDa proteins found in human liver microsomes. *Due to extensive structural homology among CYP4F2 and other human CYP4F P450s, significant cross-reactivity of anti-CYP4F+ IgG with CYP4F3a, CYP4F3b, CYP4F11 and CYP4F12 is observed.* A cross-reaction with a 70 kDa non-P450 protein is also sometimes noted, depending upon the liver sample being tested. Antibody purity has been established by denaturing SDS-PAGE, giving two protein bands with $M_R$ of 50 kDa and 25 kDa as visualized Coomassie blue staining, which correspond to the heavy and light chains, respectively, of rabbit IgG.

### ♦ Reconstitution of Lyophylized Product and Storage

Store the lyophylized product at 0-5°C. For Western blotting, reconstitute by adding 1 ml of PBS/50% glycerol to one vial of lyophilized IgG (1 mg) and mix vial gently until powder dissolves. Upon reconstitution, the solution can be stored at -20°C, as the presence of glycerol will prevent freeze/thaw cycles. Anti-CYP4F+ IgG solutions without glycerol should be also be stored at -20°C but subjected to freeze/thaw cycles as seldom as possible.



**A B C D E F G**

**Immunochemical Characteristics of Anti-CYP4F+ IgG**

Lane A = Liver Microsomes from Subject K (15 µg)
Lane B = rCYP4F2 expressed in *Sf9* insect cell lysates (0.5 µg)
Lane C = rCYP4F3b expressed in *Sf9* insect cell lysates (0.5 µg)
Lane D = rCYP4F11 expressed in *Sf9* insect cell lysates (0.5 µg)
Lane E = rCYP4F12 expressed in *Sf9* insect cell lysates (1.0 µg)
Lane F = rCYP4F3a expressed in *T. ni* insect cells (0.5 µg)
Lane G = Liver Microsomes from Subject L (15 µg)

### ♦ Use for Western Blotting

Incubate blots overnight with 2.5-5.0 µg rabbit anti-human CYP4F+ IgG IgG/ml of appropriate blocking solution. After washing to remove unbound antibody, incubate with 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.

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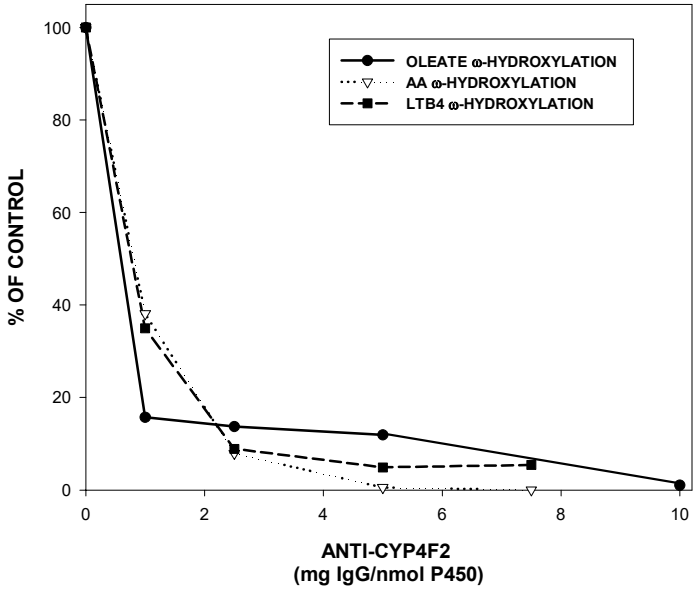
## ♦ Use for Immunoinhibition

Incubation of anti-human CYP4F+ IgG with human liver microsomes at a ratio of 1.7 mg IgG/mg microsomal protein before reaction initiation will typically give 90% inhibition of exemplary CYP4F-catalyzed reactions [e.g., arachidonate or leukotriene B4 (LTB4) ω-hydroxylation; see below]. The methodology for conducting P450 immunoinhibition assays is given in the PROTOCOLS section.




Antibodies to human CYP4F+ had a marked inhibitory effect on arachidonate, oleate and LTB4 ω-hydroxylation by liver microsomes from subjects UC9603, UC9407 and UC9209, respectively. The other P450 antibodies tested failed to effect these CYP4F-catalyzed reactions (data not shown). The values shown denote the average of triplicate determinations that differed from each other by less than 15%. As depicted, maximal inhibition of microsomal w-hydroxylation of arachidonate, oleate and LTB4 was achieved at an anti-CYP4F+ IgG:P450 ratio of 5.0 mg/nmol.

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