Antiserum was developed in rabbits using purified recombinant human CYP2C19 as immunogen. The whole IgG fraction was purified from antiserum using caprylic acid/ammonium sulfate fractionation. Anti-human CYP2C19 IgG is provided as a powder after lyophilization 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-human CYP2C19 IgG reacts extensively with its immunogen as well as with CYP2C9 (56 kDa) in human liver microsomes whereas cross-reactivity with CYP2C8 (52 kDa) is much less extensive. Reactivity of anti-human CYP2C19 with CYP2C proteins in liver microsomes from other animal species has not been characterized.

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, reconstitute by adding 1 ml of PBS/50% glycerol to one vial of lyophylized IgG (1 mg) and mix vial gently until powder dissolves. After reconstitution, solution can be stored at -20°C, as the presence of glycerol will prevent freeze/thaw cycles. Anti-CYP2C19 IgG solutions without glycerol should be also be stored at -20°C but subjected to freeze/thaw cycles as seldom as possible.

**Use for Western Blotting**
Incubate blots overnight with 2.5 - 5.0 µg rabbit anti-human CYP2C19 IgG/ml of appropriate blocking solution. After washing to remove unbound CYP2C19 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 CYP2C19 IgG with human liver microsomes at a ratio of 5 mg IgG/nmol microsomal P450 (1.7 mg IgG/mg microsomal protein) before reaction initiation will typically give 70-90% inhibition of exemplary CYP2C19-catalyzed reactions (e.g., omeprazole 5'-hydroxylation or S-mephenytoin 4'hydroxylation; see attached). Methodology for conducting P450 immunoinhibition assays is given in the PROTOCOLS section.
INHIBITION OF OMEPRAZOLE (OMP) HYDROXYLATION AND S-MEPHENYTOIN (S-MEPH) 4'-HYDROXYLATION IN HUMAN LIVER MICROSOMES BY ANTI-CYP2C19

Antibodies to human CYP2C19 had pronounced inhibitory effects on high-affinity OMP 5'-hydroxylation (Panel A) as well as S-MEPH 4'-hydroxylation (Panel B) by human liver microsomes. Ketoconazole (5 µM) was included in the OMP reaction mixtures to minimize CYP3A4 participation and to prevent conversion by this P450 of 5-hydroxyomeprazole to omeprazole sulfone (Karam et al, Drug Metab Dispos 24:1081-1087, 1996). As depicted, optimal inhibition (74%) of liver microsomal OMP hydroxylation was achieved at an anti-CYP2C19 IgG:P450 ratio of 5.0 mg/nmol while optimal inhibition (98%) of microsomal S-MEPH hydroxylation was achieved at an anti-CYP2C19 IgG:P450 ratio of 2.5 mg/nmol.