Antiserum was developed in rabbits using purified recombinant human CYP2D6 as immunogen. The whole IgG fraction was purified from antiserum using caprylic acid/ammonium sulfate fractionation. Anti-human CYP2D6 IgG is provided 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. Anti-human CYP2D6 IgG reacts with only its corresponding 49 kDa immunogen in human liver microsomes. Cross-reactivity with homologous CYP2D proteins in rat and mouse liver microsomes has not been determined.

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 lyophilized 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-CYP2D6 IgG solutions without glycerol should 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 CYP2D6 IgG/ml of appropriate blocking solution. After washing to remove unbound CYP2D6 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 CYP2D6 IgG with human liver microsomes at a ratio of 10 mg IgG/nmol microsomal P450 (3.4 mg IgG/mg microsomal protein) before reaction initiation will typically give 80% inhibition of an exemplary CYP2D6-catalyzed reaction (e.g., dextromethorphan O-demethylation). Methodology for conducting P450 immunoinhibition assays is given in the Protocols section.
Panel A shows that of the P450 antibodies tested, anti-CYP2D6 elicited the most extensive inhibition of DXM O-demethylase by liver microsomes from human subject 9409 (61% inhibition at 5 mg IgG/nmol P450). CYP3A4 antibodies also had an inhibitory effect (39% inhibition at 5 mg IgG/nmol P450) on the conversion of DXM to DXO, as predicted by Yu and Haining (Drug Metab Dispos 29:1514-1520, 2001). Maximal inhibition (79%) of microsomal DXM metabolism in this human subject was achieved at an anti-CYP2D6 IgG:P450 ratio of 10 mg/nmol (Part B).