Antiserum was developed in rabbits using purified recombinant human CYP2E1 as immunogen. The whole IgG fraction was purified from antiserum using caprylic acid/ammonium sulfate fractionation. Anti-human CYP2E1 IgG is provided either 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) or as a solution in the same buffer.

**Specificity and Purity**
Specificity has been determined by Western blotting (see below). Anti-human CYP2E1 IgG reacts with only its corresponding 55 kDa immunogen in human liver microsomes. The antibody also recognizes the homologous CYP2E1 proteins in rat and mouse liver microsomes. Specificity with whole liver homogenates or S-9 fractions 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 Lyophilized Product and Storage**
Store lyophylized product at 0-5°C. Reconstitute by adding 1 ml of PBS/50% glycerol to one vial of lyophilized IgG (1 mg) and mix vially gently until powder dissolves. After reconstitution, solution can be stored at -20°C, as the presence of glycerol will prevent freeze/thaw cycles. Anti-CYP2E1 IgG solutions without glycerol should also be stored at -20°C but subjected to freeze/thaw cycles as seldom as possible.

**Immunoreactivity of anti-CYP2E1 IgG with human liver proteins.**
- Lane A = Liver microsomes from Subject A (15 µg)
- Lane B = Purified CYP2E1 (0.1 µg)
- Lane C = Purified CYP4A11 (0.1 µg)
- Lane C = Liver microsomes from Subject B (15 µg)

**Use for Western Blotting**
Incubate blots overnight with 2.5 - 5.0 µg rabbit anti-human CYP2E1 IgG/ml of appropriate blocking solution. After washing to remove unbound CYP2E1 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 CYP2E1 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 80-90% inhibition of an exemplary CYP2E1-catalyzed reaction (e.g., chlorzoxazone 6-hydroxylation). Methodology for conducting P450 immunoinhibition assays is given in the Protocols section.