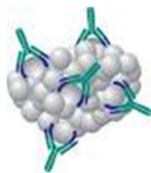


CYP450-GP



PRODUCT NUMBER Hu-A002

ANTI-CYP2E1 IgG

Polyclonal Antibody Developed in Rabbits, IgG Fraction

LOT RaBB/B#2-7

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 μ M thimerosal (added as a preservative) or as a solution in the same buffer.

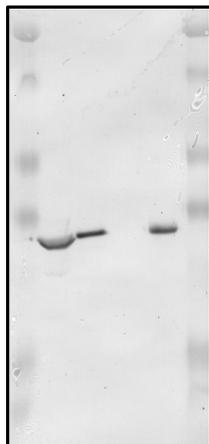
◆ **Specificity and Purity**

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 lyophilized product at 0-5°C. For Western blotting, 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 lyophilized IgG and mixing gently until powder dissolves. The solution can then 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) at 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-CYP2E1 IgG with human liver proteins

Lane A = Liver microsomes from Subject A (15 μ g)

Lane B = Purified CYP2E1 (0.1 μ g)

Lane C = Purified CYP4A11 (0.1 μ g)

Lane D = Liver microsomes from Subject B (15 μ g)

◆ **Use for Western Blotting**

Incubate blots overnight with 2.5 - 5.0 μ g 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**

Pre-incubation of anti-CYP2E1 IgG with human liver microsomes at a ratio of 2.0 mg IgG/mg microsomal protein (5 mg IgG/nmol microsomal P450) 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.

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