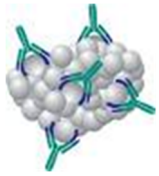
CYP450-GP



PRODUCT Hu-A005P

ANTI-HUMAN CYP3A4 Peptide IgG

Polyclonal Antibody Developed in Rabbits, IgG Fraction

LOT RaK-M/B#3-5

Antiserum was developed in rabbits using as immunogen a 21-mer peptide (VKRMKESRLEDTQKHRVDFLQ) corresponding to human CYP3A4 residues 253 to 273. The whole IgG fraction was purified from antiserum using caprylic acid/ammonium sulfate fractionation. Anti-human CYP3A4 peptide 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.

♦ Specificity and Purity

Specificity has been determined by Western blotting. Anti-human CYP3A4 peptide IgG reacts exclusively with its corresponding 51.5 kDa antigen and does not cross-react with either CYP3A5 or CYP3A7 in human liver microsomes. 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-CYP3A4 peptide IgG solutions without glycerol should be also be stored at -20°C but subjected to freeze/thaw cycles as seldom as possible.

Reaction of Anti-CYP3A4 Peptide IgG with Human Liver Proteins

Lane A = Liver microsomes from Subject E (10 µg)
Lane B = Liver microsomes from Subject F (10 µg)
Lane C = Liver microsomes from Subject G (10 µg)
Lane D = Purified CYP3A4 (0.1 µg)
Lane E = Purified CYP2E1 (0.1 µg)

♦ Use for Western Blotting

Incubate blots overnight with 5-10 µg rabbit anti-human CYP3A4 peptide IgG/ml of appropriate blocking solution. After washing to remove unbound CYP3A4 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

Anti-CYP3A4 peptide IgG exhibits *negligible* inhibition of CYP3A4 catalytic acitivity, unlike the antibody prepared against the full-length protein (Hu-A005). This lack of metabolic inhibition by the CYP3A4 peptide antibody holds true upon its incubation with liver microsomes at ratios of 8.5 mg IgG/mg microsomal protein.

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