E131 Polyclonal Antibody-Based Immunoassay for α-Fetoprotein

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α-Fetoprotein(AFP) has been a useful marker in screening and/or monitoring patients with hepatocellular carcinoma, gonadal germ cell tumor, gastric carcinoma and neural tube defects. We developed an immunodiagnostic method for the measurement of AFP in human. AFP was isolated from amniotic fluid using an isolation procedure consisting of affinity chromatography and a preparative polyacrylamide gel electrophoresis. The antibody directed against AFP was raised in rabbits. Double immunodiffusion and Western blotting methods showed that the antiserum was highly specific, reacting with only AFP-containing samples. Standard curve was obtained by using purified AFP and specific antiserum. The assay sensitivity was 2 ng of AFP/ml of human serum and the working range was 0~600 ng/ml. The within-assay and between-assay coefficient of variance(CV) was 4.3% and 7.2%, respectively. The value of Kd determined from Scatchard analysis was 0.27×10^{-9}.

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Targeting of Prostaglandin Endoperoxide H Synthases

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Prostaglandin endoperoxide H synthases-1 and -2 (PGHS-1 and PGHS-2) are integral membrane proteins associated with the lumenal surface of the endoplasmic reticulum (ER) and the nuclear envelope(NE). The C-terminal sequences of PGHSs end in -Ser/Pro-Thr-Glu-Leu (-S/PTEL), a seqeunce similar to one of the known ER targeting sequences, -Lys-Asp-Glu-Leu. Previous immunofluorescence studies had indicated that both native ovine (o) PGHS-1 and oPGHS-1 mutants with modified or deleted -PTEL sequences are localized primarily in the ER, when the proteins were expressed for 24-40 hr following transient transfection of *cos*-1 cells. However, in characterizing an oPGHS-1 mutant lacking 15 amino acids from the C-terminus (ΔCT15 oPGHS-1), we found that when this mutant was expressed for a shorter period (18 hr) following transfection, the enzyme was concentrated near the nucleus in what appeared to be the Golgi apparatus. Similar results were observed when the -P/STEL mutants of oPGHS-1 and human PGHS-2 prepared previously were retested using the 18 hr post transfection expression time.