Alpha Fetoprotein ELISA Kit

Cat. No. 99-0065

Size: 96 Tests

For Research Use Only

Lot No.

The Alpha Fetoprotein ELISA Kit is an enzyme-linked immunosorbent sandwich assay for quantitative detection of human Alpha Fetoprotein (AFP) in human serum or plasma.

INTRODUCTION

Alpha fetoprotein (AFP) is a sialic acid glycoprotein with a molecular weight of 70 kDa that consists of 590 amino acid residues.[1] It has a single polypeptide chain with three repeating domains and it is structurally related to albumin.[2] In normal tissue, AFP antibody intensely stains fetal liver, gastrointestinal tract, and yolk sac.[3]

After birth serum AFP concentration decreases rapidly, and by the second year of life and thereafter only traceable amounts are normally detected in serum. Elevation of serum AFP to abnormally high values occurs in several malignant diseases, including non-seminomatous testicular cancer and primary hepatocellular carcinoma. Elevated serum AFP concentrations are also observed in pregnant women.

REAGENTS AND MATERIALS PROVIDED

Microwell strips coated with AFP monoclonal antibody (12 x 8 wells)
Standard: 6 vials (0.7 mL each), Ready to use
Enzyme Conjugate: 1 vial (12 mL), Ready to use
TMB Substrate: 1 vial (12 mL), Ready to use
Stop Solution: 1 vial (12 mL), Ready to use
20X Wash Buffer Concentrate: 1 vial (25 mL)

MATERIALS REQUIRED BUT NOT PROVIDED

5 mL and 10 mL graduated pipettes, beakers, flasks, and cylinders
10 µL to 1,000 µL adjustable single channel micropipettes with disposable tips
50 µL to 300 µL adjustable multichannel micropipette, disposable tips, and reservoir
Microwell strip reader capable of reading at 450 nm

STORAGE

2° - 8°C

PRINCIPLE OF TEST

This ELISA kit is based on a direct solid phase sandwich ELISA method. Samples and diluted anti AFP-HRP conjugate are added to the wells coated with the monoclonal antibody to AFP beta subunit. AFP in the serum binds to antibody coated on the well, and then the anti-AFP-HRP antibody binds to AFP. Unbound proteins are washed off. Upon the addition of the substrate, the intensity of color is proportional to the concentration of AFP in the samples. A standard curve is prepared by plotting color intensity to the concentration and the AFP.

PREPARATION OF REAGENTS

20X Wash Buffer Concentrate: To prepare working wash buffer, add the contents of the bottle to 475 mL of distilled water. Store at room temperature.

PROCEDURE

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.
1. Place the desired number of coated strips into the holder
2. Pipet 50µL of AFP standards, controls, and samples into appropriate wells.
3. Pipet 100µL of enzyme conjugate into each well.
4. Cover the plate and incubate for 60 minutes at room temperature.
5. Remove the incubation mixture from all wells. Wash wells with wash buffer for three times. Blot on absorbent paper towels.
6. Add 100µL of TMB substrate into each well.

7. Incubate for 10 minutes at room temperature.
8. Add 50µL of stop solution into each well. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within **15 minutes** after adding the stop solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:
1. Check AFP standard value on each standard vial. This value might vary from lot to lot.
2. To construct the standard curve, plot the absorbance for the AFP standards (vertical axis) against its concentration in ng/mL (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Use the absorbance for controls and each unknown sample to determine the corresponding concentration of AFP from the standard curve.

PERFORMANCE CHARACTERISTICS

1. Correlation with a reference ELISA kit:

A total of 125 sera were tested by this PSA ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.9	1.2	-15.6

2. Precision

Intra-assay

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Coefficient of Variation (%)
High	16	204	11	5.39
Normal	16	142	9	6.33
Low	16	20	1.5	7.50

Inter-assay

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Coefficient of Variation (%)
High	10	210	14	6.66
Normal	10	144	12	8.30
Low	10	22	2	9.09

3. Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	0.098	0.125	0.348 ng/mL

4. Recovery

Known quantities of AFP were added to a serum that contained a low concentration of AFP.

Expected Value (ng/mL)	Recovered (ng/mL)	Percent of Recovery
247	242	98
75	78	104
27	24	88

5. Linearity

Three different patient samples were diluted with the “0” calibrator to 1:2, 1:4 and 1:8. AFP values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follow:

erum	Original Value	Percentage of Recovery		
		1:2	1:4	1:8
1	33	93	97	102
2	110	102	107	98
3	244	110	98	94

REFERENCES:

1. Smith CJP, Kelleher PC. *Biochem Biophys Acta* 650:1-32, 1980.
2. Morinaga T, et al. *PNAS* 80:4604-8460, 1983.
3. Nayak NC, Mital I. *Am J Pathol* 86:359-374, 1977.

www.invitrogen.com
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288
E-mail: techsupport@invitrogen.com

PI990065 (Rev 10/08) DCC-08-1089