

CALDON BIOTECH INC. ALPHA- FETOPROTEIN (AFP) ELISA

Catalog No. AF064T
(96 tests)

INTENDED USE

The CALDON BIOTECH INC (CBI), AFP ELISA kit is used for the quantitative measurement of AFP in human serum or plasma.

SUMMARY AND EXPLANATION

Alpha fetoprotein (AFP) is a glycoprotein with a molecular weight of approximately 70,000 daltons. AFP is normally produced during fetal and neonatal development by the liver, yolk sac, and in small concentrations by the gastrointestinal tract. After birth, serum AFP concentrations decrease rapidly, and by the second year of life and thereafter only trace amounts are normally detected in serum. Elevation of serum AFP to abnormally high values occurs in several malignant diseases, most notably nonseminomatous testicular cancer and primary hepatocellular carcinoma. In the case of nonseminomatous testicular cancer, a direct relationship has been observed between the incidence of elevated AFP levels and the stage of disease. Elevated AFP levels have also been observed in patients diagnosed with seminoma with nonseminomatous elements, but not in patients with pure seminoma. In addition, elevated serum AFP concentrations have been measured in patients with other noncancerous diseases, including ataxia telangiectasia, hereditary tyrosinemia, neonatal hyperbilirubinemia, acute viral hepatitis, chronic active hepatitis, and cirrhosis. Elevated serum AFP concentrations are also observed in pregnant women. Therefore, AFP measurements are not recommended for use as a screening procedure to detect the presence of cancer in the general population.

PRINCIPLE OF THE TEST

The AFP is a direct solid phase sandwich ELISA method. The samples and diluted anti-AFP-HRP conjugate are added to the wells coated with Mab to beta subunit. AFP in the patient's serum binds to anti-AFP Mab on the well and the anti-AFP second antibody then binds to AFP. Unbound protein and HRP conjugate are washed off by wash buffer. 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 relating color intensity to the concentration of the AFP.

MATERIALS PROVIDED

1. Microwell strips coated with AFP MAb (12x8x1 wells). Total of 96 wells.
2. AFP Standard: 6 vials (0.7 mL each). Ready to use.
3. AFP Enzyme Conjugate: 1 bottle (12 mL). Ready to use
4. TMB Substrate: 1 bottle (12 mL). Ready to use.
5. Stop Solution: 1 bottle (8 mL). Ready to use.
6. 10X Wash Concentrate: 50 mL.

STORAGE AND STABILITY

1. Store the kit at 2 - 8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. This test kit is designed for in vitro diagnostic use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that serum samples be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and

temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

(vertical axis) versus the AFP standard concentrations in mIU/mL (horizontal axis) on a linear graph paper. Draw the best curve through the points.

- Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENTS PREPARATION

10X Wash Buffer Concentrate: To prepare working wash buffer, add the contents of the bottle to 450 ml of distilled water. Store at room temperature.

ASSAY 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 50 µL of AFP standards, control and patient's sera.
3. Add 100 µL of Enzyme Conjugate to all wells.
4. Cover the plate and incubate for 60 minutes at room temperature (18 - 26°C).
5. Remove liquid from all wells. Fill wells with working wash buffer. Wash three times. Blot on absorbent paper towels.
6. Add 100 µL of TMB substrate to all wells.
7. Incubate for 10 minutes at room temperature.
8. Add 50 µL of stop solution to all wells. Shake the plate gently to mix the solution.
1. Read absorbance on ELISA Reader at 450 nm within 20 minutes after adding the stopping solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

- Check AFP standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
- To construct the standard curve, plot the absorbance for the AFP standards

EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for AFP may be used as initial guideline ranges only:

AFP Normal Range = Less Than 20 ng/ml

LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

PERFORMANCE CHARACTERISTICS

1. Correlation with a Reference ELISA kit:

A total of 125 sera were tested by this 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%
Normal	16	204	11	5.39
Low	16	142	9	6.33
High	16	20	1.5	7.50

Inter-assay

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Coefficient of Variation%
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Normal	10	210	14	6.66
Low	10	144	12	8.30
High	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)	Percentage 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 follows:

Serum	Original Value (ng/mL)	Percentage of Recovery		
		1:2	1:4	1:8
1	33	93	97	102
2	110	102	107	98
3	244	110	98	94

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