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# Alpha-Fetoprotein (AFP) ELISA

Catalog No. AF237T (96 tests)

## INTENDED USE

The Calbiotech AFP ELISA Kit is intended for the quantitative measurement of AFP in human serum. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

## SUMMARY AND EXPLANATION

AFP is a major plasma glycoprotein produced by the fetal liver, yolk sac, and gastrointestinal tract. Research focuses on its role in development, regulation of gene expression, glycoprotein function, and its potential as a molecular marker in developmental and hepatic biology studies.

## PRINCIPLE OF THE TEST

This AFP ELISA kit is a solid phase sandwich assay method, based on a streptavidin-biotin principle. The standards, samples and the biotinylated Anti-AFP antibody reagent are added into designated wells, coated with Streptavidin. Endogenous AFP in the patient's serum binds to the antigenic site of the biotinylated Anti-AFP antibody. Simultaneously, the biotinylated antibody is immobilized onto the wells through the high affinity Streptavidin-Biotin interaction. Unbound protein and excess biotin conjugated antibody are washed off by wash buffer. Upon the addition of the Peroxidase (HRP) conjugated Anti-AFP antibody reagent, a sandwich complex is formed, the analyte of interest being in between the two highly specific antibodies, labeled with Biotin and HRP. Unbound protein excess enzyme conjugated antibody reagent is washed off by wash buffer. Upon the addition of the substrate, the intensity of color developed is directly 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 | | 96 TESTS |
|---|---|---|
| 1. | Microwell coated with Streptavidin | 12x8x1 |
| 2. | AFP Standards: 6 vials (ready to use) | 6 x 0.5 mL |
| 3. | AFP Controls: 2 vials (ready to use) | 2 x 0.5 mL |
| 4. | Anti-AFP Enzyme Conjugate: 1 bottle (ready to use) | 12 mL |
| 5. | Anti-AFP-Biotin Reagent: 1 bottle (ready to use) | 12 mL |
| 6. | TMB Substrate: 2 bottles (ready to use) | 2 x 8 mL |
| 7. | Stop Solution: 1 bottle (ready to use) | 12 mL |
| 8. | 20X Wash Concentrate: 2 bottles | 2 x 25 mL |

## MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

## 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.
4. Do not expose test reagents to heat, sun, or strong light.

**WARNINGS AND PRECAUTIONS**

Potential biohazardous materials:

1. The standards 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. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
5. 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.

**SPECIMEN COLLECTION HANDLING**

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

**REAGENTS PREPARATION**

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

**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. Pipette 25 µl of AFP standards, control and patient's sera.
3. Add 100 µl of Anti-AFP-Biotin Reagent to all wells and mix for 20-30 seconds.
4. Cover the plate and incubate for 30 minutes at room temperature (20-25° C).
5. Remove liquid from all wells. Wash wells three times 300 µl with 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of the Anti-AFP- Enzyme conjugate to all wells. Cover and incubate for 30 minutes.
7. Remove liquid from all wells. Wash wells three times 300 µl with 1X wash buffer. Blot on absorbent paper towels.
8. Add 100 µl of TMB substrate to all wells.
9. Incubate for 15 minutes at room temperature.
10. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
11. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping 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. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for the AFP standards (vertical axis) versus the AFP standard concentrations in ng/ml (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

**Example of a Standard Data**

| | OD 450 nm | Conc. ng/mL |
|---|---|---|
| **Std 1** | 0.020 | 0 |
| **Std 2** | 0.072 | 5 |
| **Std 3** | 0.281 | 25 |
| **Std 4** | 0.462 | 50 |
| **Std 5** | 1.878 | 250 |
| **Std 6** | 2.447 | 500 |

**LIMITATIONS OF THE TEST**

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.