IVD

Alpha-fetoprotein (AFP) ELISA

Catalog No. E0203-2

96 tests

INTENDED USE

The Autobio AFP ELISA kit is intended for the quantitative determination of alpha-fetoprotein (AFP) concentration in human serum.

INTRODUCTION

Alpha-fetoprotein (AFP) is a glycoprotein with a molecular weight of approximately 70,000 daltons. AFP is produced mainly by the fetal yolk sac and fetal liver and to a lesser extent by the fetal gastrointestinal tract and kidneys[1].

Elevation of serum AFP to abnormally high values occurs in several malignant diseases, most notably non-seminomatous testicular cancer and primary hepatocellular carcinoma. Approximately 70% of patients with primary hepatocellular carcinoma show elevated levels of AFP[2]. In the case of testicular teratoma a direct relationship has been observed between incidence of elevated AFP levels and the stage of disease[3]. No increased AFP levels are found in testicular seminomas[4]. The application of AFP measurement to the management of carcinoma patients has been well documented[5].

In addition, elevated serum AFP concentrations have been measured in patients with other non-cancerous 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 assay is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-AFP antibody for solid phase (microtiter wells) immobilization and another mouse anti-AFP monoclonal antibody (MAb) in the antibody-enzyme (horseradish peroxidase) conjugate reagent. The test specimen is added to the AFP antibody coated microtiter wells. Then the anti-AFP MAb labeled with horseradish peroxidase (HRP) is added. If human AFP is present in the specimen, it will combine with the antibody on the well and the conjugate will bind immunologically to the AFP on the well, resulting in the AFP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed with wash fluid to remove unbound conjugates. Substrate solution and chromogen solution are added and incubated, resulting in the development of a blue color. The color development is terminated with the addition of stop solution, and the color is changed to yellow and is measured spectrophotometrically at the wavelength of 450 nm. The concentration of AFP is directly proportional to the color intensity of the test sample.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: microplate with murine anti-AFP MAb coated wells (1 plate, 96 wells)
2. Enzyme Conjugate Reagent: HRP labeled murine anti-AFP MAb in stabilizing buffer (1 vial, 7.2 mL)
3. Reference Standards: 10, 20, 50, 100, 200 and 400 ng/mL AFP in human serum with preservatives. (6 vials, 1 ml/ea)
4. Wash Fluid Concentrate: PBS-Tween (1 vial, 8.0 mL, 62.5×)
5. Substrate Solution: hydrogen peroxide (1 vial, 7.4 mL)
6. Chromogen Solution: tetramethylbenzidine (TMB) (1 vial, 7.4 mL)
7. Stop Solution: 1.0 M H_2SO_4 (1 vial, 7.4 mL)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micropipettes and multichannel micropipettes of appropriate volume (the use of accurate pipettes with disposable plastic tips is recommended)
2. Distilled water
3. Vortex mixer
4. Absorbent paper or paper towel
5. Graph paper
6. Incubator
7. Disposable reagent troughs
8. Instrumentation
   a) Automated microplate strip washer
   b) Microplate reader
   or
   c) Fully automated microplate processor

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2 – 8℃ upon receipt. The test kit may be used throughout the expiration date of the kit (1 year from the date of manufacture). Refer to the package label for the expiration date.
2. Reference Standards should be stored sealed at 2 – 8℃. The remaining Reference Standards opened should be used within 30 days and be frozen at -20℃ for long term storage. Avoid multiple freeze-thaw cycles of Reference Standards.
3. Microplate after first use should be kept in a sealed bag with desiccants to minimize exposure to damp air. Other opened components will remain stable for at least 2 months, or until the expiration date, whichever is earlier, provided it is stored as prescribed above.

SPECIMEN COLLECTION, PREPARATION, TRANSPORT AND STORAGE

1. Serum is the recommended sample type for this assay. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
2. Collect all blood samples observing universal precautions for venipuncture.
3. Allow samples to clot for 1 hour before centrifugation.
4. Avoid grossly hemolytic, lipemic or turbid samples.
5. Prior to use, specimens should be capped and stored up to 48 hours at 2 – 8℃. Specimens going to be stored or transported for more than 48 hours must be stored frozen (- 20℃ or lower). Avoid multiple freeze-thaw cycles. After thawing, ensure specimens are thoroughly mixed and brought to room temperature before being assayed.

PRECAUTIONS AND WARNINGS

1. for *in vitro* diagnostic use only
2. This package insert must be fully understood prior to operation. The operation must be stringently in accordance with the instruction for use.
3. Handling of reagents, serum specimens should be in accordance with local safety procedures.
4. Reference Standards contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported. Nevertheless, the Reference Standards and components containing animal substances should still be treated as potentially infectious.
5. Avoid any skin contact with all reagents. Stop Solution contains H_2SO_4, in case of contact, wash thoroughly with water.
6. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18 - 25°C) before use. All reagents should be mixed through gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute the Wash Fluid Concentrate with 500 mL of distilled water prior to use.

IMPORTANT NOTES

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. It is recommended that no more than 32 wells be used for each assay run, if manual pipetting is used, since pipetting of all Reference Standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
4. Replace caps on reagents immediately. Do not switch caps.
5. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 50μL of each Reference Standard and specimens into appropriate wells.
2. Dispense 50 μL of Enzyme Conjugate Reagent to each well.
3. Incubate at 37℃ for 30 minutes.
4. Remove the incubation mixture by emptying plate content into a waste container. Rinse and empty the microtiter wells 5 times with the diluted Wash Fluid. The volume of each well is about 350 μL. Dry the plate by striking it sharply onto absorbent paper or paper towels after the last wash cycle. Alternatively, wash it in an automated microplate strip washer 5 times.
5. Dispense 50 μL of Substrate Solution into each well, then 50 μL of Chromogen Solution into each well. Gently mix and incubate for 5 minutes at room temperature in the dark.
6. Stop the reaction by adding 50 μL of Stop Solution to each well.
7. Gently mix for 10 seconds to ensure that the blue color completely turns to yellow.
8. Read absorbance at a wavelength of 450 nm in a microplate reader within 10 minutes.

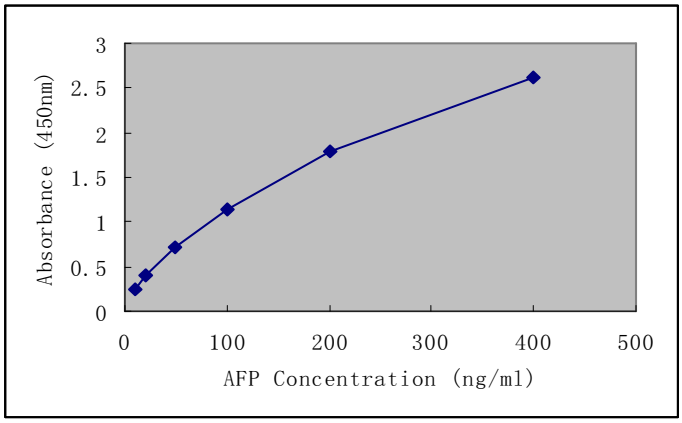
CALCULATION OF RESULTS

1. Calculate the mean values from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance for each sample, determine the corresponding AFP concentration in ng/mL from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

EXAMPLE OF STANDARD CURVE

A typical standard curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

AFP (ng/mL)	Absorbance (450 nm)
10	0.251
20	0.403
50	0.726
100	1.143
200	1.802
400	2.624



REFERENCE NORMAL RANGE

Each laboratory should establish its own reference normal range. These values are given only for guidance.

Sample Numbers	124
Average Value（ng/ml）	8.20
Standard Deviation ( )	5.90
Normal Range (+2σ, ng/ml)	20.0

PERFORMANCE CHARACTERISTICS

1. Analytical Sensitivity

The sensitivity of the assay, defined as the concentration of AFP equivalent to the mean absorbance of 20 replicates of the zero Reference Standard plus two standard deviations, is typically 2.5 ng/mL.

2. Specificity

No interference was detected with the performance of Autobio AFP ELISA upon addition of massive amounts of the following substances to a human serum pool.

Interferents	**Concentration**
human albumin	100 mg/mL
CEA	5 g/mL
HCG	100 IU/mL
PRL	10 g/mL

3. Precision

3.1. Intra-assay precision

Intra-assay precision was determined by assaying 20 replicates of control, , respectively.

Serum	**Number**	**Mean**	**SD**	**RSD（%）**
control	20	24.36	1.43	5.87

3.2. Inter-assay precision

Inter-assay precision was determined by assaying 1 serum pools in duplicate across 20 separate runs, using a standard curve constructed for each run.

Serum	**Number**	**Mean**	**SD**	**RSD（%）**
control	20	23.87	2.35	9.84

4. High Dose Hook Effect

No significant interference was observed with AFP values up to 12,000 g/L. However, since patients with advanced hepatocellular carcinoma may show extremely high levels, false low results due to a high dose hook effect may be seen in specimens from these patients. In order to avoid reporting misleadingly low results due to a hook effect at higher concentrations, particularly in patients for whom markers are being measured for the first time, or when very high AFP values may be expected, it is recommended to assay specimens at two dilution rates (i.e. neat and diluted 1:100 with normal human serum).

5. Accuracy

For 175 samples in the range of 10 ng/mL to 400 ng/mL, the relationship between the Autobio AFP ELISA and the Roche Elecsys® assay is described by the equation:

Reference	Number of Specimens	Least Square Regression Analysis	Correlation coefficient
Roche（ECLIA）	175	y = 0.9118x + 0.9042	0.932

LIMITATIONS

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis.
3. Serum samples demonstrating gross lipemia, gross hemolysis,or turbidity should not be used with this test.
4. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

SYMBOLS

LOT	**BATCH CODE**
	USE BY
	MANUFACTURER
Σ	**CONTAINS SUFFICIENT FOR <n> TESTS**
IVD	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**
8 °C 2 °C	**TEMPERATURE LIMITATION**
REF	**CATALOGUE NUMBER**
	CONSULT INSTRUCTIONS FOR USE

REFERENCES

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for orders and inquiries, please contact

AUTOBIO DIAGNOSTICS CO., LTD.
ADD: No.87 Jingbei Yi Road, National Eco & Tech Development Area, Zhengzhou , China 450016
Tel: +86-371-67985313 Fax: +86-371-67985804
Web: www.autobio.com.cn