

AFP - ELISA

Cat. #: BL-26-E Version 050215-BL

Tests: 96 Wells

Enzyme immunoassay for the quantitative determination
of Alpha-Fetoprotein in serum.

Not be used for risk calculation of Trisomie 21

CLINICAL RELEVANCE

Alpha-fetoprotein (AFP) is a glycoprotein with a molecular weight of approximately 70 KD(1). AFP is normally produced during fetal and neonatal development by the liver, yolk sac, and in small concentrations by the gastrointestinal tract (2). After birth, serum AFP concentrations decrease rapidly, and by the second year of life and thereafter only trace amounts are normally detected in serum (3).

Elevation of serum AFP to abnormally high values occurs in several malignant diseases (4-7), 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 (8-9). Elevated AFP levels have also been observed in patients diagnosed with seminoma with nonseminomatous elements, but not in patients with pure seminoma (6,8,10-11).

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 (12-15). Elevated serum AFP concentrations are also observed in pregnant women (16-17). Therefore, AFP measurements are not recommended for use as a screening procedure to detect the presence of cancer in the general population.

Intended use

The main clinical applications of measurements of AFP are found in the monitoring of cancer following treatment. Although AFP measurement may also be of clinical interest in monitoring of pregnancy when applied to serum or amniotic fluid, this assay is not intended to be used as a screening parameter in Trisomie 21.

Cancer

- ❖ Hepatocellular carcinoma
- ❖ Teratocarcinomas and embryonal cell carcinoma of testis and ovaries
- ❖ Yolk sac tumor
- ❖ Other cancers (less than 5 %).

Viral diseases

- ❖ Acute hepatitis (usually < 100 IU/ml)
- ❖ Chronic active hepatitis (usually < 100 IU/ml).

PRINCIPLE

AFP ELISA KIT is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a AFP molecule. An aliquot of patient sample containing endogenous AFP is incubated in the coated well with enzyme conjugate, which is an anti-AFP antiserum conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off

with water. The amount of bound peroxidase is proportional to the concentration of AFP in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of AFP in the patient sample.

REAGENTS

1. **Microtiter wells**,
Wells coated with anti-AFP monoclonal antibody, 96 wells.
2. **Reference Standard Set**, 5 vials (lyoph.)
0, 10; 40; 80; 160 IU/ml (1IU/ml = 1,21ng/ml).
see „Reagent Preparation“
3. **Enzyme Conjugate** - Anti-AFP antiserum conjugated to horseradish peroxidase, 11 ml.
4. **Substrate Solution** - TMB, 11 ml.
5. **Stop Solution** - 0,5M H₂SO₄, 6 ml.

Note: Additional Zero Standard for Sample dilution available on request.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. A microtiterplate reader (450±10 nm)
2. Precision micropipettes with disposable tips for 10, 25, 50 and 100 µl.
3. Standard refrigerator.
4. Absorbent paper.
5. bidist. water

Note: It is very important to use bidist. water!

STORAGE CONDITIONS

When stored at 2° to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Enzyme-Conjugate, Substrate Solution, Standards and Zero Standard must be stored at 2° to 8°C.

Microtiter wells must be stored at 2° to 8°C. Once the foilbag has been open care should be taken to close it tightly again. The immuno-reactivity of the coated microtiter wells is stable for approx. 6 weeks in the broken, but tightly closed bag containing the dessicant.

WARNINGS AND PRECAUTIONS FOR USERS

1. **CAUTION:** Test methods are not available which can offer complete assurance that Hepatitis B virus, Human Immunodeficiency Virus (HIV/HTLV-III/LAV), or other infectious agents are absent from the reagents in this kit. Therefore, all human blood products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation, where it exists (e.g., USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories," 1984).
2. Avoid contact with *Stop Solution* 0,5 M H₂SO₄. It may cause skin irritation and burns.
3. Replace caps on reagents immediately. Do not switch caps.
4. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
5. Do not pipette reagents by mouth.
6. For in vitro diagnostic use only.
7. Do not mix or use components from kits with different lot numbers.

SPECIMEN COLLECTION AND PREPARATION

1. Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Avoid hemolysis.

ATTENTION! This kit is for use with samples without additives only.

2. Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying. Specimen held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

REAGENT PREPARATION

Reference Standards: Reconstitute the lyophilized contents of the standard vial with 0.5 ml bidist. Water.

Note: The reconstituted standards are stable for 2 months at 2-8°C. For longer storage freeze at -20°C.

PERFORMANCE OF THE ASSAY

GENERAL REMARKS:

1. All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
2. Once the test has been started, all steps should be completed without interruption.
3. Use new disposable tips for each specimen.
4. Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
5. The present AFP kit is adjusted to give an absorption for standard 4 of 1.200 to 2.000 within 10 minutes at room temperature (22°C). If that absorption value is above the upper performance limit of your microtiterplate spectrophotometer or lower than 1.200, you can reduce or extend the incubation time of the final enzymatic formation of color accordingly. As a general rule the enzymatic reaction is linearly proportional to time and temperature. This makes interpolation possible for fixed physico-chemical conditions.

PROCEDURAL NOTE

1. Manual Pipetting: It is recommended that no more than 32 wells be used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. Automated Pipetting: A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes.
3. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.

SPECIMEN DILUTION COMMENTS

If in an initial assay, a serum specimen is found to contain more than 160 IU/ml AFP, the specimens can be diluted 10-fold with *Zero Standard* and reassayed as described in Assay Procedure.

ASSAY PROCEDURE

1. Secure the desired number of *Microtiter Wells* in the holder.
2. Dispense 25 µl AFP *Standards* (0; 10; 40; 80; 160 IU/ml), controls and serum specimen **with new disposable tips** into appropriate wells.
3. Dispense 100 µl Anti-AFP *Enzyme-Conjugate* into each well.
4. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for 30 minutes at room temperature.
6. Briskly shake out the contents of the wells.
7. Rinse the wells 5 times with Aqua dest.

8. Strike the wells sharply on absorbent paper to remove residual water droplets.
9. Add 100 µl of *Substrate Solution* to each well.
10. Incubate for 10 minutes at room temperature.
11. Stop the enzymatic reaction by adding 50 µl of *Stop Solution* to each well.
12. Read the OD at 450±10 nm with a microtiterplate reader.

Final Reaction Stability

It is recommended that the wells be read within 10 minutes following step 11.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of reference standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in IU/ml with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration of AFP in IU/ml from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Any diluted samples must be further converted by the appropriate dilution factor.

EXPECTED VALUES

1. It is strongly recommended that each laboratory should determine its own normal and abnormal values.
2. The lower limit of AFP concentration in normal serum is less than 1IU/ml. The upper limit is about 10 IU/ml.
3. Indicative values of AFP in maternal serum during pregnancy.

<u>Weeks of pregnancy</u>	<u>AFP in serum(IU/ml)</u>
6 - 9	0 - 12
10 - 12	10 - 30
13 - 15	13 - 60
16 - 18	16 - 93
19 - 21	32 - 139
22 - 24	56 - 224
25 - 27	95 - 357
28 - 30	135 - 435
31 - 33	141 - 423
34 - 36	121 - 379
37 - 40	93 - 320

CLINICAL IMPORTANCE

1. Maternal serum containing above 2.5 times the normal median for weeks 16 to 18 of pregnancy was detected in 88% of cases of anencephaly and in 79% of cases of open spina bifida.
2. The concentration of AFP in hepatocellular carcinoma and germ cell tumor varies from the normal range up to several million IU/ml. After surgical resection, the serum AFP may drop to normal range or somewhat above it.
3. AFP may occur in serum of patients with diseases other than hepatocarcinoma or embryonal carcinoma of the testes, such as neonatal hepatitis and nonhepatic neoplasms.

PERFORMANCE CHARACTERISTICS

1. Specificity

Human serum albumin was tested for cross-reactivity in the assay:

Human Serum Albumin	Produced Color Intensity Equivalent to AFP in serum (IU/ml)
1.25 g/dl	<1.0
2.50 g/dl	<1.0
5.00 g/dl	<1.0
10.00 g/dl	<1.0

2. Hook Effect

No hook effect was observed in this test up to 1000 IU/ml of AFP.

3. Sensitivity

The minimum detectable concentration of AFP by this assay is estimated to be 1.0 IU/ml.

QUALITY CONTROL

Good laboratory practice requires that controls are run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. Controls containing azide should not be used.

LIMITATION OF PROCEDURES

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. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances.

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PROCEDURE FLOW SHEET AFP ELISA BL-26-E

Description	Standard Sample	Enzyme- Conjugate		Substrate Solution		Stop- Solution		Results IU/ml
	μl	μl		μl		μl		
Standard 0	25	100	Mix for 10 seconds Incubate for 30 minutes at RT Rinse the wells 5 times with water	100	Incubate for 10 minutes at RT	50	Read the OD at 450 nm with a microtiter-plate-reader	0
Standard 1	25	100		100		50		10
Standard 2	25	100		100		50		40
Standard 3	25	100		100		50		80
Standard 4	25	100		100		50		160
Sample 1	25	100		100		50		
Sample 2	25	100		100		50		
Sample 3	25	100		100		50		
Sample 4	25	100		100		50		
Sample 5	25	100		100		50		