Alpha Feto Protein (AFP) ELISA Kit

96 Tests Kit
Enzyme Immunoassay for the
Quantitative Determination of AFP
Concentration in Human Serum/Plasma
(For In Vitro Diagnostic Use Only)
Catalogue No. PT-AFP-96

PISHTAZ TEB DIAGNOSTICS

Introduction

Alpha-Feto Protein (AFP) is a 70,000 kDa glycoprotein molecule which is closely related to human albumin both genetically and structurally. AFP is produced initially by the fetal yolk sac in small quantities and then in large quantities by the fetal liver as the yolk sac degenerates. The peak concentration in the fetal serum is approximately 3,000,000 ng / ml, and it is reached at this level within 9 weeks gestation. The concentration then declines steadily to about 20,000 ng / ml at the end of term. Maternal AFP is first detectable at 5 ng / ml in serum at about 10 weeks gestation. The concentration increase about 15 % per weeks to a peak of approximately 180 ng / ml at about 25 weeks. Measurement of AFP concentration in maternal serum in the second trimester of pregnancy and amniotic fluid used widely to screen for prenatal open neural tube defect (NTD) in the fetus. In addition determination of AFP concentration is helpful in the diagnosis and management of patients with primary hepatoma, yolk sac carcinoma and testicular and presacral teratocarcinomas.

Test Principle

The AFP Quantitative Test Kit is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes an anti-AFP antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-AFP antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react with the solid phase antibodies, after incubation and washing, the enzyme conjugate will be added, resulting in the sandwich formation of AFP between solid phase and conjugated antibodies. After second wash step a solution of chromogen-substrate is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of AFP is directly proportional to the color intensity of the test sample.

Materials provided with the kit

1. Antibody coated wells (1 plate, 96 wells): Microtiter wells coated with monoclonal anti AFP
2. Enzyme conjugate (1 vial, 12 ml): Monoclonal anti AFP labeled with HRP in buffer containing protein as stabilizer and Thiomerosal as preservative, ready to use
3. Standards set (1 ml /vial): Contain 0.0(2 ml), 5, 20, 50, 100 and 200 ng/ml of AFP diluted in buffer containing protein as stabilizer and 0.05% Thiomerosal as preservative.
4. Low control serum (1 ml / vial): Contains certain amount of AFP diluted in buffer containing protein as stabilizer and 0.05% Thiomerosal as preservative.
5. High control serum (1 ml / vial): Contains certain amount of AFP diluted in buffer containing protein as stabilizer and 0.05% Thiomerosal as preservative.
6. Assay buffer (1 vials, 12 ml): Contains phosphate buffer solution with protein as stabilizer and Kathon CG as preservative, ready to use.
7. Chromogen substrate reagent (1 vials, 12 ml): Contains tetramethyl benzidine and hydrogen peroxide, ready to use solution.
8. Wash solution (1 vial, 50 ml): Contains phosphate buffer salt solution with 0.05 %

Tween 20 as detergent, pH = 6, concentrated (20X).
9. Stop solution: (1 vial, 12 ml): 1 molar hydrochloric acid solution, pH < 1.
10. Cardboard sealer.

Materials required but not provided

- Precision pipettes: 50 µl and 100 µl
- Disposable pipette tips.
- Distilled water.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter well reader.

General Information

1. Do not mix kit reagents from different batch/lot numbers. All kit components must be used only in their original kit.
2. All reagents obtained from human sources are negative for HBs Ag, HCV and HIV antibodies. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

Storage Conditions

1. Kit should be stored at 2-8 ^{o}C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.

Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate (maximum 48 hours) the specimen immediately and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20ºC (maximum 30 days). Avoid freeze-thaw of specimen during storage.

Reagents Preparation

1. All reagents should be allowed to reach room temperature (22-28ºC) before use.
2. Working wash solution: dilute concentrated wash solution 1:20 with distilled water before use.

Test Procedure

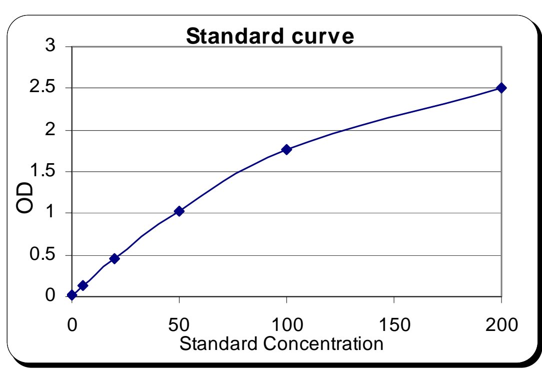
1. Secure the desired number of coated wells in the holder and keep the remaining with desiccants in tightly closed special bag.
2. Dispense 50 µl of standard, serum control and specimen in appropriate wells in duplicate.
3. Add 100 µl of assay buffer into each well and shake wells gently for 15 seconds to mix well.
4. Cover the microtiter wells with cardboard sealer firmly. Leave wells for 30 minutes at room temperature (22-28ºC).
5. Remove the sealer and take out wells contents by flicking the microplate into a waste container.
   Rinse and flick the microtiter wells 5 times (each time with 300 µl of working wash solution). Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
6. Add 100 µl of Anti-AFP-HRP conjugate into the wells.
7. Seal the plate with cardboard sealer again. Leave wells for 30 minutes at room temperature (22-28ºC).
8. Repeat step 5
9. Dispense 100 µl of chromogen/ substrate solution into the microplate wells.
10. Incubate the microplate wells at room temperature and dark for 15 minutes, to develop color.
11. Stop the reaction by adding 100 µl of stop solution to the microplate wells.
12. Measure absorbance at 450 nm by ELISA reader. (Use 630 nm filter as reference filter if it’s available).

Result Calculation

1. Calculate mean absorbance value of standards and samples at 450 nm. (Use 630 nm filter as reference filter if it's available).
2. Construct a point to point 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. Use the mean absorbance value for each sample; determine the corresponding concentration of AFP in ng/ml from the standard curve.

Example of Standard curve

Standards ng/ml	OD
0	0.02
5	0.14
20	0.46
50	1.02
100	1.76
200	2.50



Note: All absorbances shown in above curve and table are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

Expected Values

It is important for each laboratory to establish the normal range limits. The following reference range from 126 healthy persons should be considered as a guideline only:

Reference Interval ng / ml

Mean	SD	Reference Inteval (90 % CI)
2.2	2.4	0.2 – 8.5

Gestational weeks	Median of AFP in maternal sera ng / ml
15	22
16	26
17	31
18	35
19	41
20	44
21	52

Performance Characteristics

1. Minimum Detection Limit

The minimum detectable concentration of AFP by this assay is estimated to be 1 ng/ml.

2. Test Precision

Three serum samples with different concentrations of AFP were repeatedly tested. Results are shown in table 1 and 2:

Table 1: Intra-assay

No.	No. of tests performed	Means ng / ml	SD ng / ml	CV %
1	24	1.9	0.11	5.7
2	24	33	1. 55	4.7
3	24	130	6.00	4. 6

Table 2: Inter-assay

No.	No. of tests* performed	Means ng / ml	SD ng / ml	CV %
1	10	1.92	0.16	8.3
2	10	32.3	2.40	7.5
3	10	131	7.70	5.9

*Each test has been run in duplicate

3. Test Recovery

The certain amount of AFP was added into 4 different serums with known concentrations of AFP and then their recovery were determined. The results shown below:

Table 3: Test recovery

No.	AFP Level ng / ml	AFP Added ng / ml	Exp. ng /ml	Obs. ng / ml	Rec. (%)
1	3.2	5	4.1	3.8	93
1	3.2	20	11.6	11	95
1	3.2	100	51.6	49	95
2	18	5	11.5	12.5	108
2	18	20	19	18	95
2	18	100	59	56	95
3	62	5	33.5	35	107
3	62	20	36	34	94
3	62	100	81	85	105
4	167	5	86	81	94
4	167	20	93.5	101	108
4	167	100	133.5	125	93

Exp.: Expected, Obs.: Observed,
Rec.: Recovery

4. Test Linearity

To verify test linearity, three different serum samples with known AFP concentration were diluted sequentially by zero standard. Then the serums were tested by AFP ELISA test. The results and serum recovery were determined considering dilution factor.

Table 4: Test linearity

		Recovery (%)				
No	AFP(ng/ml) undiluted specimen	1:2	1:4	1:8	1:16	1:32
1	184	104	98	89	106	93
2	74	99	100	92	95	90
3	45	102	95	98	110	108

5. Hook effect

To rule out possible hook effect occurrence, the AFP assay was done on serums with high concentration of AFP (up to 5 µg/ml) and no “hook effect” was seen.

References

1- Johnson, P.J, (2002) Tumor Markers in Primary Malignancies of the liver. In “Tum or Markers: Physiology, pathobiology, technology and clinical applica- tions”, ed. Dimandis E.P. AACC Press, Washington
pp 269-276.

2- Stenman, U-H and alfthan, H. (2002) Markers for Testicular Cancer. In “Tum or Markers: Physiology, pathobiology, technology and clinical applications”, ed. Dimandis E.P. AACC Press, Washingon
pp 269-276.

3- Christiansen, M., Hogdall, C.K., Anderson, J.R. and Norgaard-Pederson,
B. (2001) Alpha-fetoprotein in plasma and serum of healthy adults: preanalytical, analytical, and biological sources of variation and construction of age-dependent reference intervals. Scand J Invest
61: 205-216.

4-Trape, J., Botargues, J.M.,Porta, F., Ricos, C.,Badal, J.M., Salinas,
R., Sala, M., and Roca, A. (2003) Reference change value for a-Fetoprotein and its application in early detection of hepatocellular carcinoma in patients with hepatic disease. Clin Chem 49(7): 1209-1211

5- Nustad, K., Paus, E., Kierulf, B., and Bormer, O.P. (1998) Specificity and affinity of 30 monoclonal antibodies against Alpha-Fetoprotein.
Tumor Bio 19: 293-300.

6- National Committee for Clinical Laboratory Standards, Evaluation of Precision Performance of Clinical Chemistry Devices. Approved Guideline EP5-A (1999).

AFP Test Procedure

Step 1

Reagent	**Standard**	**Control Serum**	**Sample**
Standard	50 µl	None	None
Control Serum	None	50 µl	None
Sample	None	None	50 µl
Assay Buffer	100 µl	100µl	100 µl

Gently mixed for 15 seconds and cover the microplate wells with cardboard sealer tightly and incubate them for 30 minutes at room temperature.

Step 2

Remove plate cover and discard contents of the wells. Wash the microplate wells for 5 times according to test manual.

Step 3

Anti AFP – HRP conjugate	100 µl	100 µl	100 µl

Cover the microplate wells again with cardboard sealer and incubate them for 30 minutes at room temperature.

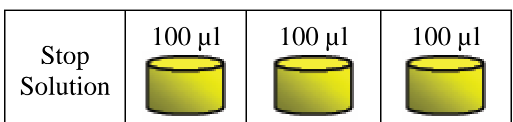
Step 4

Remove the microplate wells cover and remove contents by flicking of the wells info waste container wells 5 times according to test manual.

Chromogen -substrate solution	100 µl	100 µl	100 µl

Incubate wells for 15 minutes at room temperature in dark.

Step 5



Stop Solution	100 µl	100 µl	100 µl

Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).