Prestige Diagnostics
Clinical Diagnostics & Point of Care Products

AFP (Alpha-fetoprotein) Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIAAFP1	AFP Elisa	96 Tests

Intended Use:

AFP Elisa is intended to be used for the quantitative determination of AFP in Human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

AFP is a glycoprotein with a molecular weight of approximately 70K daltons. AFP is normally produced during fetal and neonatal development by the liver, yolksac 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 the serum. Elevated of serum AFP to abnormally high levels occurs in several malignant diseases, most notably nonseminomatous testicular cancer and primary hepatocellular carcinoma. In the case of non seminomatopus 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 AFP concentrations have been measured in patients with other noncancerous diseases including ataxia talangiectasia, herediary tyrosinemia, neonatal hyperbilirubinemia, acute 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.

The AFP Elisa is based on a solid phase EIA. The assay system utilizes one anti-AFP antibody for solid phase immobilization and another mouse monoclonal anti-AFP antibody in the antibody enzyme conjugate solution. The test sample is allowed to react simultaneously with the antibodies resulting in the AFP molecules being sandwiched between the solid phase and enzyme linked antibodies. After 60 minute incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes resulting in the development of a blue colour. The colour development is stopped with the addition of 2N HCl, and the colour is changed to yellow and measured at 450nm. The concentration of AFP is directly proportional to the intensity of colour developed.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with monoclonal anti AFP antibodies. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable for 2 months at 2-8°C.
AFP Calibrators	6x1ml	6 vials containing AFP at concentrations of 0.0, 5, 20, 50, 150 and 300 ng/ml made up in a human serum matrix. Reference against WHO 72/225 THE EXACT CONCENTRATIONS ARE PROVIDED ON THE VIAL LABEL. CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. Ready to use. Once open stable for 1 month at 2-8°C.
Zero Buffer	1x12ml	1 vial containing Zero buffer. Once open stable for 2 months at 2-8°C.
Enzymatic Conjugate	1x18ml	1 vial containing 18ml of HRP labelled monoclonal Anti AFP antibodies in Buffered saline. Once open, stable for 2 months at 2-8°C.
Wash Buffer Concentrate (50X)	1x15ml	PBS-Tween at pH 7.4. 50X concentrate. The concentrate must be diluted with 735ml of distilled water before use. Once diluted it is stable at room temperature for two months.
Substrate Solution	1x12ml	Mixture of TMB and Hydrogen Peroxide solution. Ready to use. Once open, stable for 2 months at 2-8°C.
Stop Solution	1x12ml	Diluted Sulfuric acid solution (1M) Ready to use. Once open, stable for 2 months at 2-8°C.

Plastic Sealable bag, IFU and Cardboard plate covers.

Materials required but not provided:

Distilled water, Vortex mixer, Micropipettes, Incubator, Microplate Reader and Microplate washer.

Specimen Collection:

Serum should be prepared from whole blood specimen obtained by acceptable medical techniques. The kit is for use with serum samples without additives only.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination. Place unused wells in zip-lock bag provided and return to 2-8°C, under which conditions the wells will remain stable for 2 months, or until the labelled expiry date, whichever is earlier. Seal and return all the other unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the labelled expiry date, whichever is earlier.

Procedure:

Reagent preparation:

1. Bring all reagents to room temperature (18-22°C) prior to use.
2. Dilute the wash buffer concentrate with 735ml of Distilled water (yielding a total volume of 750ml). Once diluted the wash solution is stable for 2 months at room temperature. Mix well before use.

STEP 1

Preparation: Remove the number of wells required and number each well for the assay series.

STEP 2

Addition of Samples and calibrators: Add 50ul of Calibrators and Samples to each well.

STEP 3

Addition of Zero Buffer: Add 100ul of the Zero Buffer solution to each well. Shake the plate for 10 seconds to ensure that the added components are well mixed.

STEP 4

Incubation: Cover the plate with the plate cover and incubate for 30 minutes at room temperature (18-22°C).

STEP 5

Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 5 times with diluted washing buffer of 350ul. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of Enzyme Conjugate: Add 150ul of the Enzyme Conjugate to each well. Shake the plate for 5 seconds to ensure that the added components are well mixed.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 30 minutes at room temperature (18-22°C).

STEP 8

Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 5 times with diluted washing buffer of 350ul. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 9

Addition of the Substrate: Add 100ul of Substrate Solution to each well. Mix gently for 5 seconds.

STEP 10

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at room temperature. Ensure that the incubation is done in the dark.

STEP 11

Stopping the Reaction: Add 100ul of the Stop solution into each well and mix gently. Shake the plate to mix till the solution changes to yellow from blue.

STEP 12

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader within 15 minutes of adding the Stop Solution. Note down the absorbances.

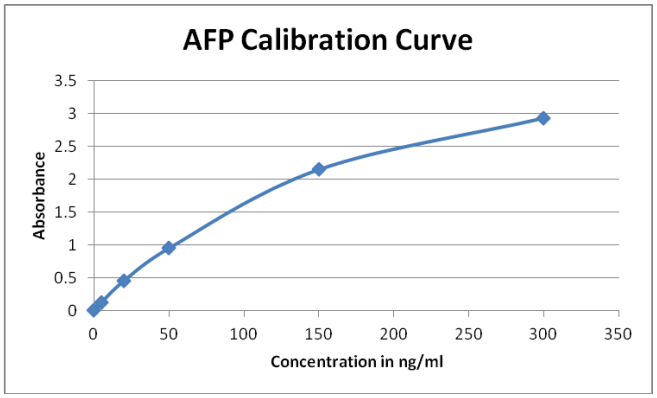
Note: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the absorbance in Y axis and Concentration in ng/ml in X axis.
- Draw a point to point curve through the plotted points on a linear graph paper.
- To determine the concentration of an unknown sample, locate the absorbance of the sample on the Y axis and find the intersecting point on the curve. Read the concentration from the X axis by dropping a line from the intersecting point of the absorbance on the curve.

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	0.012	0.0 ng/ml
CAL B	0.127	5.0 ng/ml
CAL C	0.455	20.0 ng/ml
CAL D	0.952	50.0 ng/ml
CAL E	2.150	150.0 ng/ml
CAL F	2.932	300.0 ng/ml



Expected Values:

In high risk patients, AFP values between 100 and 350 ng/ml suggest a diagnosis of hepatocellular carcinoma, and levels over 350 ng/ml usually indicate the disease. Approximately 97% of the healthy subjects have AFP levels less than 8.5 ng/ml. It is recommended that each laboratory establish its own normal ranges.

Performance Characteristics:

1. **Intra assay Precision:**

Panel	Data no.	Mean	SD	CV%
1	24	31.04 ng/ml	1.45	4.66 %
2	24	126.8 ng/ml	5.20	4.10 %
3	24	270.8 ng/ml	12.93	4.78 %

2. **Inter assay Precision:**

Panel	Data no.	Mean	SD	CV%
1	24	30.58 ng/ml	1.88	6.1 %
2	24	125.1 ng/ml	7.08	5.7 %
3	24	268.3 ng/ml	14.06	5.2 %

3. **Sensitivity:**

The minimum detectable concentration of AFP by this assay was found to be 2 ng/ml.

Linearity:

A patient serum was serially diluted with 0 ng/ml standard in a linearity study. The average recovery was 102.2%

Sample A			
Dilution	Expected	Observed	% Recovery
Sample undil	271.2 ng/ml	271.2 ng/ml	
2x	135.6 ng/ml	137.12 ng/ml	101.1 %
4x	67.8 ng/ml	69.45 ng/ml	102.4 %
8x	33.9 ng/ml	35.16 ng/lm	103.7 %
16x	16.95 ng/ml	17.20 ng/ml	101.5 %
32x	8.48 ng/ml	9.31 ng/ml	109.9 %
Average Recovery: 102.2%			

Method Comparison:

Method comparison between this assay and a commercially available assay yielded the following data:

N=79, Correlation Coeff: 0.985, Slope: 1.038, Intercept: 0.729

Mean values: This assay: 55.12 ng/ml and comparator: 52.24 ng/ml

Recovery:

Various patient serum samples of AFP levels were mixed and assayed in duplicate. The average recovery was 101.1%

Expected conc ng/ml	**Observed Conc ng/ml**	**% Recovery**
92.7	94.2	101.6
77.63	79.31	102.2
23.24	23.10	99.4
156.18	159.23	102.0
206.06	210.10	102.0
148.94	150.52	101.1
149.73	148.97	99.50
Average Recovery: 101.1%		

Cross Reactivity:

The following materials were tested for cross reactivity and the results are as follows:

Antigens	Concentration	Equivalent AFP	% Cross reactivity
HCG	400 IU/ml	0.0 ng/ml	0.0 %
PAP	1000 ng/ml	0.0 ng/ml	0.0 %
PSA	1000 ng/ml	0.0 ng/ml	0.0 %
CEA	1000 ng/ml	0.0 ng/ml	0.0 %

No High dose hook effect was observed.

References:

1. Abelev G.I. Alpha-fetoprotein as a marker of embryo-specific differentiation in normal and human tissue. Transplant Rev 1974; 20:3-37.
2. Hiral H. Alpha-fetoprotein. In Chu TM ed., Biochemical markers for cancer. New York: Marcel Dekker, 1982: 23-59.
3. Chan DW, Miao Y C. Affinity chromatographic separation of Alpha-fetoprotein variants: Development of a mini column procedure and application to cancer patients. Clin Chem, 1986; 32:2143-2146.
4. Sell LS. Cancer markers of the 1990s. Clin Lab Med 1990; 10: 1-37.
5. Hirai H, Nishi S, Watabe et al. Some chemical experimental and clinical investigations on alpha fetoprotein. In: Hitai H, Miyaji T. eds. Alphs-Fetoprotein and hepatoma. Gann Manogr 1973: 14: 19-34.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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