

Instruction Manual No. M-0500

Human Alpha-Fetoprotein (AFP)

ELISA KIT Cat. No. 0500

For Quantitative Determination of AFP In Serum

For In Vitro Research Use Only

Human Alpha-Fetoprotein (AFP) ELISA KIT # 500

Kit Contents: (reagents for 96 tests)

C o m p o n e n t s	C a t #
Anti-hAFP coated microwell strip plate (96 wells). Ready-to-use	5 0 1
AFP Standard A , 0 IU/ml/Sample. Diluent, 11 ml	5 0 2
AFP Standard B , 3 IU/ml; 0.50 ml	5 0 3
AFP Standard C , 10 IU/ml; 0.50 ml	5 0 4
AFP Standard D , 25 IU/ml; 0.50 ml	5 0 5
AFP Standard E , 100 IU/ml; 0.50 ml	5 0 6
AFP Standard F , 200 IU/ml; 0.50 ml	5 0 7
Anti-hAFP- HRP Conjugate , 11 ml	5 0 8
HRP substrate Solution , 11 ml	T M B - 5 0 0
Wash Buffer (100X) ; 10 ml, dilute 1:100 with distilled water	W - 1 0 0
Stop solution , 10 ml	T - 1 0
Complete Instruction Manual	M - 5 0 0

Notes: AFP Stds are calibrated against WHO IS 72/225 (1 IU=1.21 ng AFP).

INTRODUCTION

AFP is a glycoprotein with a mol wt of ~65-70 kDa. It has approx. 4% carbohydrates. During fetal development, AFP is secreted at high levels and then drops to very low levels in adult life. AFP reappears in serum at high levels in malignant diseases of hepatocellular, testicular, nonseminomatous origin, and occasionally other entodermal origin. AFP may be slightly elevated or persisted in patients with large hepatic metastases or viral hepatitis. AFP measurement is widely accepted as tumor marker and for monitoring the therapeutic effectiveness of hepatocellular and other cancer.

AFP concentration is also high in the amniotic fluid during early stages of pregnancy. AFP levels decline in the later part of the pregnancy. Elevated amniotic AFP levels are indicative of open neural tube defects (spina bifida or anencephaly) and in several fetal hemolytic diseases, omphlocele, esophageal artresia, congenital nephrosis, intrauterine death or fetal bleeding into the amniotic fluid. For diagnostic purpose, ultrasonography and acetylcholine esterase measurement should be performed in conjunction with AFP.

ADI's AFP ELISA kit is a very sensitive assay for the measurement of AFP in human serum.

PERFORMANCE CHARACTERISTICS

2. PRECISION

Intra-assay precision:

Three serum samples (mean AFP concentrations 18, 81.6, 167 IU/ml) were run in 10 replicates. The samples showed good intra-assay precision with %CV of 4.09, 3.5, and 7.8, respectively.

Inter-assay precision:

Three serum samples (15, 77, and 173.0 IU/ml) were run in duplicate in 10 independent assays. The samples showed good inter-assay precision (5.00-11.3 %CV).

3. RECOVERY

A known amount of AFP (10, 50, and 100 IU/ml) was added to three patient sera (with original AFP concentrations of 16, 53, and 138 IU/ml) and the total AFP concentrations measured. The assay showed excellent mean recoveries of about 96% (range 96-101%).

4. LINEARITY

Six different patient samples (with original AFP concentrations of 36, 66, 88, 105, 136, and 206 IU/ml) were diluted (1:2, 1:5, and 1:10) with the zero standard and their final AFP values determined. The samples showed excellent mean recoveries of about 99% (range 88-112%).

5. SPECIFICITY

The specificity of AFP ELISA kit was determined by measuring interference from high concentrations of human albumin and human Vitamin D binding protein. No interference was detected.

6. HIGH DOSE HOOK EFFECT

AFP concentration of 50,000 IU/ml did not cause any hook effect.

7. SPECIES REACTIVITY

Human AFP kit has not been tested in other species (mouse, rat, human etc).

GENERAL REFERENCES : Ruoslahti E et al (1974) Transplant Rev. 20, 30-60; Silver HKB et al (1973) PNAS 70, 526-530; Brauenstein GD et al (1973) Cancer 31, 1065-1068; McIntire KR et al (1975) Cancer Res. 35, 991-996; Silver HKB et al (1974) Cancer Res. 34, 244-247; Purves LR et al (1973) Africa Gann Monograph 14, 51-66; Bosl GJ et al (1981) Cancer 47, 328.

Citations of ADI Human AFP ELISA kit

Toietta G 2003 Molecular Therapy 7, 649-658, Human Blood
Kajiyama Y 2002 Mol. Cell. Biol. 22, 6122-6130, human albumin and AFP detection by ELISA in cultured cells Hu7 Cells grown in 5% FCS

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Arrange required # of strips. Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dispense 200-300 ul of wash buffer to all wells. Mix for 5 seconds and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.

Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 1-liter). Store at 4°C until use.

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **25 µl** of standards, controls (users supplied), and serum samples into appropriate wells in *duplicate*. Immediately, dispense **100 µl** of antibody enzyme conjugate into each well.
3. Mix gently for 5-10 seconds, cover the plate and **incubate at room temp. for 60 minutes**.
4. Aspirate and wash the wells 5 times with wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
5. **Dispense 100 ul TMB substrate per well** . Mix gently, cover the plate and **incubate for 30 min at room temp.** Positive wells will develop blue color.
6. Add **50 ul** stop solution into each well (blue color turns into yellow). Read absorbance at 450 nm within 30 minutes.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision.

Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C.

Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Serum samples should be diluted with the Std./Sample diluent and amniotic fluid samples with normal saline. A 1:100 dilution is suggested for all amniotic fluid samples. Samples containing more than 200 IU/ml AFP must also be diluted and the results obtained should be multiplied by the appropriate dilution factor.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standards from the mean absorbance values of standards, control, and samples. Draw the standard curve on log-log graph paper by plotting net absorbance values of standards against appropriate AFP concentrations. Read off the AFP concentrations of the control and patient samples.

PERFORMANCE CHARACTERISTICS

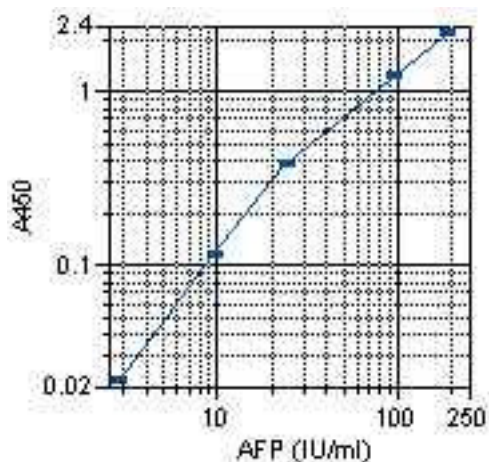
1. DETECTION LIMIT

Based on sixteen replicate determinations of the zero standard, the minimum concentration of AFP detected using this assay is 1.3 IU/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Net Abs.	Calculated Conc (mIU/l)
A1, A2	Std. A (0 IU/ml)	0.005	
B1, B2	Std. B (3 IU/ml)	0.038	
C1, C2	Std. C (10 IU/ml)	0.120	
D1, D2	Std. D (25 IU/ml)	0.287	
E1, E2	Std. E (100 IU/ml)	0.993	
F1, F2	Std. F (200 IU/ml)	1.680	
G1, G2	Sample 1	0.230	19
H1, H2	Sample 2	0.718	72

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std assay curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

AFP ELISA kit is based on sequential binding of human AFP from samples to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of AFP present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. The unknown sample values are then read-off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (25-100 μ l) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

research use only. The Control and Standards have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H₂SO₄ (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera can not be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

Reagent Preparation:

Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 1-liter).