Alpha-fetoprotein (AFP) ELISA Kit

For the Quantitative Determination of Alpha-fetoprotein (AFP) Concentrations

Catalogue Number: EL10049

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR DIAGNOSITIC USE

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S7.5 (02/15-02) AFP

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INTENDED USE

This AFP ELISA Kit is to be used for the *in vitro* quantitative determination of alpha-fetoprotein in human blood samples and cell culture supernatant. This kit is intended **FOR LABORTORY RESEARCH USE ONLY** and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

Alpha-fetoprotein (AFP) is a glycoprotein with a molecular mass of 65,000 Daltons. It is normally produced in large amounts by liver, the visceral endoderm of foetus. Alpha-fetoprotein level increases progressively and reaches a peak at the 30th week in the maternal serum. Thereafter, it decreases gradually and reduces to trace amount with a couple of years after birth. Human AFP gene is located at the chromosomal location 4q25. The expression of AFP is regulated by a large promoter P1 and three distant enhancers. AFP expression abnormality is a relatively common genetic disorder that affects intellectual development and causes other health problems. Increased AFP expression is observed in adults that developed metastasis of liver and other organs. AFP is thought to be a foetal form of serum albumin and exists in monomeric, dimeric and trimeric forms. It binds to copper, nickel, fatty acids and bilirubin. The exact function of human AFP is still under investigation. Several hypotheses have been proposed for the physiological function: regulation of cell growth, sexual differentiation, transportation of metals and other substances, interaction with cytoplasmic chaperone proteins, and protecting foetus against the maternal immunity. To cover the very large range of concentration associated with the great variety of processes, the current immunoassay is a simple tool for the detection of AFP in the range 0 – 400ng/ml.

PRINCIPLE OF THE ASSAY

This AFP enzyme-linked immunosorbent assay (ELISA) applies a technique called quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for AFP. Standards or samples are then added to the appropriate microtiter plate wells and incubated. AFP if present, will bind and become immobilized by the antibody pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove any unbound AFP and other components of sample. In order to quantitate the amount of AFP present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated monoclonal antibody specific for AFP is added to each well to "sandwich" the AFP immobilized during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP-conjugated antibodies and a TMB (3,3'5,5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain AFP and enzyme-substrate reaction will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the colour change measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of AFP in the samples, this kit includes a set standard. This allows the operator to produce a standard curve of Optical Density (O.D) versus AFP (ng/mL). The concentration of AFP in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	96 tests

1. **AFP MICROTITER PLATE** (Part EL49-1) ***96 wells***
   Pre-coated with anti-human AFP monoclonal antibody.
2. **AFP CONJUGATE** (Part EL49-2) ***15 mL***
   Anti-human AFP monoclonal antibody conjugated to horseradish peroxidase with preservative.
3. **AFP STANDARD – 400ng/mL** (Part EL49-3) ***1 vial***
   Lyophilized human AFP (WHO, 72/225) in a buffered protein base with preservative that will contain 400ng/ml after reconstitution.
4. **AFP STANDARD – 200ng/mL** (Part EL49-4) ***1 vial***
   Lyophilized human AFP (WHO, 72/225) in a buffered protein base with preservative that will contain 200ng/ml after reconstitution.
5. **AFP STANDARD– 100ng/mL** (Part EL49-5) ***1 vial***
   Lyophilized human AFP (WHO, 72/225) in a buffered protein base with preservative that will contain 100ng/ml after reconstitution.
6. **AFP STANDARD – 50ng/mL** (Part EL49-6) ***1 vial***
   Lyophilized human AFP (WHO, 72/225) in a buffered protein base with preservative that will contain 50ng/ml after reconstitution.
7. **AFP STANDARD – 20ng/mL** (Part EL49-7) ***1 vial***
   Lyophilized human AFP (WHO, 72/225) in a buffered protein base with preservative that will contain 20ng/ml after reconstitution.
8. **AFP STANDARD – 10ng/mL** (Part EL49-8) ***1 vial***
   Lyophilized human AFP (WHO, 72/225) in a buffered protein base with preservative that will contain 10ng/ml after reconstitution.
9. **AFP STANDARD – 0ng/mL** (Part EL49-9) ***1 vial***
   Lyophilized buffered protein base with preservative that will contain 0ng/ml after reconstitution.
10. **ZERO BUFFER** (Part EL49-10) ***15 mL***
    Animal serum with buffer and preservative. .
11. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
    20-fold concentrated solution of buffered surfactant.
12. **SUBSTRATE A** (Part EL49-11) ***11 mL***
    Buffered solution with H_2O_2.
13. **SUBSTRATE B** (Part 30007) ***11 mL***
    Buffered solution with TMB.
14. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100µL and 50-200µL for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm).
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Wastes: Autoclave for 60 minutes at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the virus before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form, which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

HANDLING AND STORAGE

Samples be used within 24-48 hours if stored at 2-8°C, otherwise samples must stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.

- When performing the assay slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **AFP Standard:**

   a) Reconstitute AFP Standards with 0.5 mL of Deionized or distilled water. Allow the reconstituted solution to stand for at least 15 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored frozen.

ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and AFP Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards and Samples to the Microtiter Plate.*

Wells	Contents	Wells	Contents
1A, 1B	Standard 1 - **0 ng/mL** (S1)	**2A, 2B**	Standard 5 - **100mL** (S5)
1C, 1D	Standard 2 - **10 ng/mL** (S2)	**2C, 2D**	Standard 6 - **200/mL** (S6)
1E, 1F	Standard 3 - **20 ng/mL** (S3)	**2E, 2F**	Standard 7 - **400g/mL** (S7)
1G, 1H	Standard 4 - **50 ng/mL** (S4)	**2G-12H**	AFP samples

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S5	2	6	10	14	18	22	26	30	34	38
B	S1	S5	2	6	10	14	18	22	26	30	34	38
C	S2	S6	3	7	11	15	19	23	27	31	35	39
D	S2	S6	3	7	11	15	19	23	27	31	35	39
E	S3	S7	4	8	12	16	20	24	28	32	36	40
F	S3	S7	4	8	12	16	20	24	28	32	36	40
G	S4	1	5	9	13	17	21	25	29	33	37	41
H	S4	1	5	9	13	17	21	25	29	33	37	41

2. Add 20μL of Standard or Sample to the appropriate well of the antibody pre-coated wells of the Microtiter Plate and dispense 100ul of zero buffer into each well, thoroughly mix for 30 seconds. Cover and incubate for 30 minutes at 37°C

3. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μL/well/wash (range: 350-400 μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

4. Add 100μL of conjugate to each well. Cover and incubate for 30 minutes at 37°C.

5. Prepare Substrate Solution (see Preparation of Reagents) no more than 15 minutes before end of second incubation.

6. Repeat wash procedure as described in Step 3.

7. Add 100 μL Substrate Solution to each well. Cover and incubate for 10 minutes at 37°C.

8. Add 100 μL Stop Solution to each well. Mix well.

9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of AFP in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding AFP concentration (ng/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 ng/mL) or (S1) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of AFP in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding AFP concentration. If samples generate values higher than the highest standard, dilute the samples and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

3. To determine the final concentration of AFP in serum or plasma samples, the concentration read from the standard curve must be multiplied by the *dilution factor.*

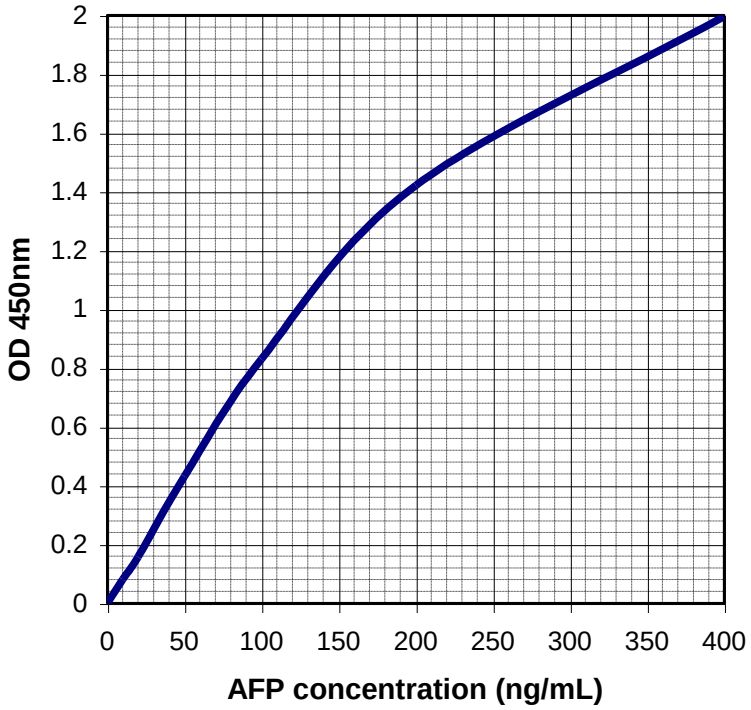
TYPICAL DATA

Results of a typical standard run of an AFP ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of *illustration only*, and should not be used to calculate unknowns. Each user should obtain their own standard curve.

EXAMPLE

The following data was obtained for a standard curve.

Standard (ng/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted
0	0.051,0.048	0.050	0.000
10	0.129,0.130	0.130	0.080
20	0.200,0.214	0.207	0.157
50	0.478,0.483	0.481	0.431
100	0.882,0.878	0.880	0.830
200	1.458,1.483	1.471	1.421
400	2.041,2.05	2.046	1.996



PERFORMANCE CHARACTERISTICS

1. **PRECISION**

a) **INTRA-ASSAY PRECISION**
To determine within-run precision, three different samples of known concentration were assayed by using 15 replicates in 1 assay.

b) **INTER-ASSAY PRECISION**
To determine between-run precision, three different samples of known concentration were assayed by replicates on 15 different assays.

	Intra-assay			***Inter-assay***		
Sample	1	2	3	1	2	3
n	15	15	15	15	15	15
Mean (ng/mL)	32.8	86.4	306	28.1	70.1	307.4
Standard Deviation (ng/mL)	1.41	3.54	6.73	1.7	3.8	21.7
Coefficient of Variation (%)	*4.3*	*4.1*	*2.2*	*6.0*	*5.4*	*7.1*

2. RECOVERY
The recovery of AFP spiked to 3 different levels in samples throughout the range of the assay in various matrices was evaluated.

Mixed serum samples	Expected(ng/ml)	Measured(ng/ml)	Recovery(%)
1	190.6	196.1	103
2	161.8	168.1	104
3	141.8	41.4	99

3. **SENSITIVITY**

The minimum detectable dose of AFP was determined by adding two standard deviations to the mean optical density value of 16 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of human AFP using a standard curve generated is 2.0 ng/mL.

4. **SPECIFICITY**

This kit exhibits no detectable cross-reactivity with CEA, PSA, HCG, CA125, CA153, and human serum albumin.

5. **CALIBRATION**

This immunoassay is calibrated against NIBSC AFP 1st international standard (code 75/225).

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