HUMAN ALPHA FETO PROTEIN (AFP) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN ALPHA FETO PROTEIN CONCENTRATIONS IN SERUM AND PLASMA

PURCHASE INFORMATION:

ELISA NAME	HUMAN AFP ELISA
Catalog No.	SK00245-02
Lot No.	
Formulation	96 T
Standard range	0.39-50 ng/mL
Sensitivity	50 pg/mL
Sample require	100 µL per well
Dilution Factor	N/A
Sample Type	Serum, EDTA Plasma
Specificity	Human AFP
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2-8 °C

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INTRODUCTION

Human Alpha FetoProtein (AFP) immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human AFP in serum and plasma. It contains natural human AFP and antibodies raised against this protein. It has been shown to accurately quantify human AFP. Results obtained with naturally occurring AFP samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human AFP.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for AFP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any AFP present is bound by the immobilized antibody. After washing away any unbound substances, an antibody biotinylated specific for AFP is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is add to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of AFP bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
AFP Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified IgG against human AFP.	**245-02-01**	**1 plate**
AFP Standard – 50 ng/vial of Human AFP in a buffered protein base with preservatives; lyophilized.	**245-02-02**	**1 vial**
Detection Antibody– 1.05 mL/vial, 10-fold Concentrate of a purified IgG biotinylated against AFP with preservatives; lyophilized.	**245-02-03**	**1 vial**
Positive Control – one vial of recombinant AFP , lyophilized	**245-02-04**	**1 vial**
Streptavidin-HRP Conjugate -120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservatives	**DB08A**	**1 bottle**
Wash Buffer - 50mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution - 11mL of 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 12 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70°C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20 °C or -70°C for up to one month. Anti Rabbit IgG-HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 12 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

Note: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.

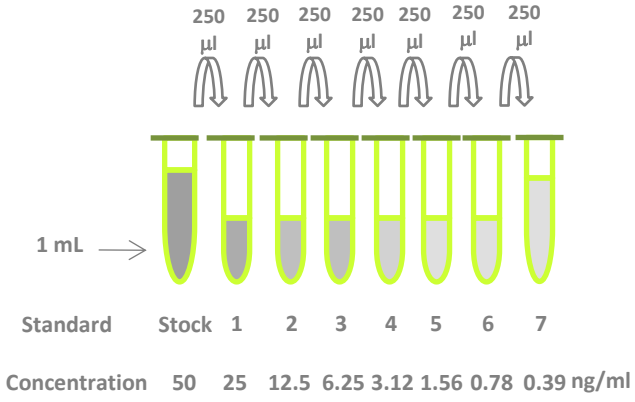
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

AFP Standard - Refer to vial label for reconstitution volume. Reconstitute the **AFP** standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 50 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of the appropriate Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **50 ng/mL** standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0 ml	50 ng/ml
# 1	250µl of stock	250µl	25 ng/ml
# 2	250µl of 1	250µl	12.5 ng/ml
# 3	250µl of 2	250µl	6.25 ng/ml
# 4	250µl of 3	250µl	3.125 ng/ml
# 5	250µl of 4	250µl	1.56 ng/ml
# 6	250µl of 5	250µl	0.78 ng/ml
# 7	250µl of 6	250µl	0.39 ng/ml



Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of the appropriate Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. ***This Detection Antibody working solution requires incubation for 2 hour at room temperature on shaker before its using.***

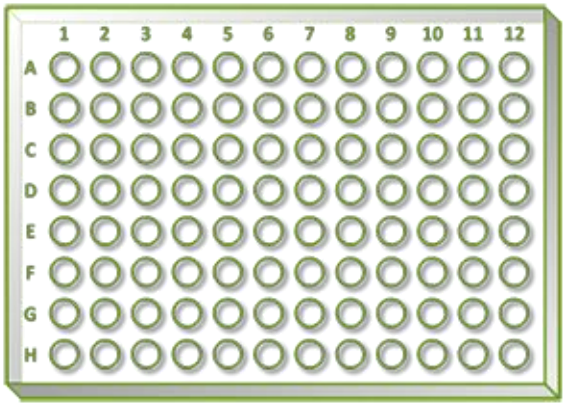
Streptavidin-HRP Conjugate - Transfer 120 µl of 100-fold concentrated stock solution to 12 ml of Dilution Buffer to prepare working solution. Note: 1 x working solution of Streptavidin HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the Positive Control with 1.5 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100 µL of Dilution Buffer to Blank wells (A2, A3).
4. Add 100 µL of Standard solution from #7 to #S (reverse order of serial dilution) (from B2, B3 to G2, G3 , G4, G5 to F4, F5), sample, or positive control (E4, E5) per well. Cover with the plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with the plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin HRP Conjugate** working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 5-10 min at room temperature. **Protect from light. Be ready to add stop solution quickly.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the AFP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.

This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Calculation of samples with a concentration exceeding that of 50 ng/mL may result in inaccurate, low human AFP levels. Such samples require further external predilution according to expected human AFP values with Dilution Buffer in order to precisely quantify the actual human AFP level.

CALIBRATION

This immunoassay is calibrated against a highly purified natural human AFP.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of AFP was 50 pg/mL.

SPECIFICITY

PROTEIN NAME	CROSS-REACTIVITY
Human AFP	100%
Human albumin	0
Human VDBP	0
Human RBP4	0
Mouse AFP	0

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED)
Blank	**0 (0.076)**
0.39	**0.035**
0.78	**0.072**
1.56	**0.150**
3.125	**0.256**
6.25	**0.460**
12.5	**0.705**
25	**1.029**
50	**1.401**

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT. Incubation of detection antibody working solution for 2 hours at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 2-5 min on the plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min