ab108631 – alpha Fetoprotein (AFP) Human ELISA Kit

Instructions for Use

An immunoenzymatic assay for the quantitative measurement of alpha Fetoprotein in Human Serum.

This product is for research use only and is not intended for diagnostic use.

Version 1 Last Updated 11 February 2019

Table of Contents

1. BACKGROUND

Abcam's alpha Fetoprotein (AFP) *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of alpha Fetoprotein in Human serum.

A 96-well plate has been precoated with anti-alpha Fetoprotein antibodies. Samples and standards and are added to the wells, where alpha Fetoprotein in the sample and standards binds to the precoated antibody. After incubation and washing, added Anti-alpha Fetoprotein-HRP conjugate binds to the antibody-alpha Fetoprotein complex. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is directly proportional to the amount of alpha Fetoprotein in the sample and the intensity is measured at 450 nm.

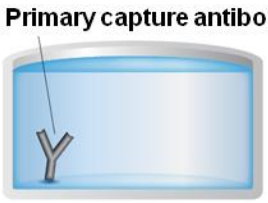
Alpha-fetoprotein (alpha Fetoprotein) is a glycoprotein with a molecular weight of approximately 70,000 Daltons. alpha Fetoprotein is normally produced during fetal and neonatal development by the liver, yolk sac, and in small concentrations by the gastrointestinal tract. After birth, serum alpha Fetoprotein concentrations decrease rapidly, and by the second year of life and thereafter only trace amounts are normally detected in serum.

Elevation of serum alpha Fetoprotein to abnormally high values occurs in several malignant diseases, most notably non-seminomatous testicular cancer and primary hepatocellular carcinoma. In the case of non-seminomatous testicular cancer, a direct relationship has been observed between the incidence of elevated alpha Fetoprotein levels and the stage of disease. Elevated alpha Fetoprotein levels have also been observed in samples with seminoma with non-seminomatous elements, but not in samples with pure seminoma.

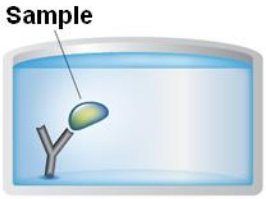
In addition, elevated serum alpha Fetoprotein concentrations have been measured in samples with other noncancerous diseases, including ataxia telangiectasia, hereditary tyrosinemia, neonatal

hyperbilirubinemia, acute viral hepatitis, chronic active hepatitis, and cirrhosis. Elevated serum alpha Fetoprotein concentrations are also observed in pregnant women. Therefore, alpha Fetoprotein measurements are not recommended for use as a screening procedure to detect the presence of cancer in the general population.

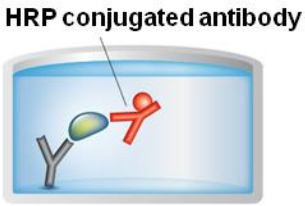
2. ASSAY SUMMARY



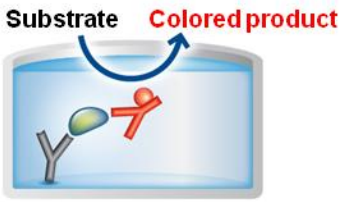
Prepare all reagents, samples and standards as instructed.



Add samples and standards to wells used. Add Zero Buffer and mix well. Incubate at room temperature.



After washing, add prepared labeled HRP-Conjugate to each well. Incubate at room temperature.



After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Anti-alpha Fetoprotein Coated Microplate (12 x 8 wells)	96 Wells	2-8°C
Stop Solution	11 mL	2-8°C
Anti-alpha Fetoprotein HRP Conjugate	18 mL	2-8°C
TMB Substrate Solution	11 mL	2-8°C
Zero Buffer	13 mL	2-8°C
alpha Fetoprotein Standard 0 – 0 ng/mL	0.5 mL	2-8°C
alpha Fetoprotein Standard 1 – 5 ng/mL	0.5 mL	2-8°C
alpha Fetoprotein Standard 2 – 20 ng/mL	0.5 mL	2-8°C
alpha Fetoprotein Standard 3 – 50 ng/mL	0.5 mL	2-8°C
alpha Fetoprotein Standard 4 – 150 ng/mL	0.5 mL	2-8°C
alpha Fetoprotein Standard 5 – 300 ng/mL	0.5 mL	2-8°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Incubator at 37°C
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer
- Absorbent paper or paper towel.

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells
- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

- All solutions are supplied ready to use

10. SAMPLE COLLECTION AND STORAGE

- The determination of alpha Fetoprotein can be performed in Human serum. Microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay. If the assay is performed on the same day of sample collection, the specimen should be kept at 2-8°C; otherwise it should be aliquoted and stored deep-frozen (-20°C). If samples are stored frozen, mix thawed samples gently for 5 minutes before testing.

  Avoid repeated freezing and thawing

11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates)

12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**

13.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3. Add 20 µL standards, control and samples into their respective wells.

13.4. Add 100 µL of Zero Buffer into each well. Mix gently for 30 seconds.

Note: Complete mixing is essential for good assay performance

13.5. Cover wells with the foil supplied in the kit and incubate at room temperature for 30 minutes.

13.6. Remove the foil, aspirate the contents of the wells and wash each well five times with 300 µL of deionized or distilled water. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. After the last wash, remove the remaining deionized or distilled water by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.

Note: Complete removal of liquid at each step is essential for good assay performance.

13.7. Add 150 µL of Anti-alpha Fetoprotein HRP Conjugate into each well. Gently mix for 10 seconds.

13.8. Incubate at room temperature for 30 minutes.

13.9. Repeat wash step 13.6

13.10. Add 100 µL TMB Reagent into each well. Gentle mix for 10 seconds.

13.11. Incubate at room temperature for 20 minutes in the dark.

13.12. Stop the reaction by adding 100 µL of Stop Solution to each well. Mix gently for 30 seconds.

Note: It is important to make sure that all the blue color changes to yellow color completely.

13.13. Measure the absorbance of the sample at 450 nm within 15 minutes of addition of the Stop Solution.

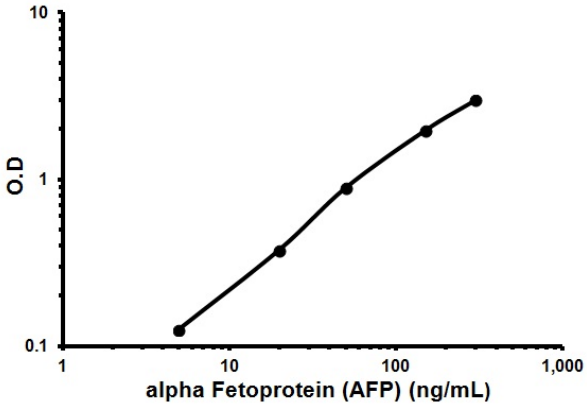
13. CALCULATIONS

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Conc. (ng/mL)	Mean O.D. (-Blank)
0	0.053
5	0.126
20	0.378
50	0.891
150	1.968
300	2.986

15. TYPICAL SAMPLE VALUES

REFERENCE VALUES-

In high-risk patients, alpha Fetoprotein 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 alpha Fetoprotein levels less than 8.5 ng/mL. It is recommended that each laboratory establish its own normal range.

SENSITIVITY –

The minimum detectable concentration of alpha Fetoprotein is estimated to be 2.0 ng/mL.

16. ASSAY SPECIFICITY

This kit detects alpha Fetoprotein in Human samples. Other species have not yet been tested with this kit.

17. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time to short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

18. NOTES

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