Product Manual

Human Alpha- Fetoprotein (AFP) ELISA Kit

Catalog Numbers

PRB- 5058	96 assays
PRB- 5058- 5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Alpha-fetoprotein (AFP) is a glycoprotein produced early in fetal development by the liver. After birth, plasma levels of AFP drop rapidly and reach normal levels within the first year. However, elevated serum AFP has been found in patients with benign liver diseases (e.g. cirrhosis, hepatitis) and various cancers (e.g. hepatocellular carcinoma, hepatoblastoma, germ cell tumors), making it a useful biomarker. AFP measurement has become an essential screening and monitoring tool for chronic liver diseases and cancers.

Cell Biolabs' AFP ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the human AFP protein. The kit has detection sensitivity limit of 150 pg/mL AFP. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and AFP samples.

Assay Principle

An anti-AFP coating antibody is adsorbed onto a microtiter plate. AFP protein present in the sample or standard binds to the antibodies adsorbed on the plate; a biotinylated anti-AFP antibody is added and binds to the antigen captured by the first antibody. Following incubation and wash steps, a streptavidin-enzyme conjugate is added and binds to the biotinylated anti-AFP antibody. Unbound streptavidin-enzyme conjugate is removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of AFP present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from purified AFP and sample concentration is then determined.

Related Products

1. PRB-5049-C: Human PSA ELISA Combo Kit (Free + Total)
2. PRB-5049-FREE: Human Free PSA ELISA Kit
3. PRB-5049-TOTAL: Human Total PSA ELISA Kit
4. PRB-5059: Human CEA ELISA Kit
5. CBA-100: CytoSelect™ 24-Well Cell Migration Assay (8µm, Colorimetric)
6. CBA-106: CytoSelect™ 96-Well Cell Migration Assay (8µm, Fluorometric)
7. CBA-110: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Colorimetric)
8. CBA-125: Radius™ 24-Well Cell Migration Assay (Microscopy)
9. CBA-126: Radius™ 96-Well Cell Migration Assay (Microscopy)
10. CBA-130: CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-AFP Antibody Coated Plate (Part No. 50581B): One strip well 96-well plate.
2. Biotinylated Anti-AFP Antibody (1000X) (Part No. 50582D): One 20 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human AFP Standard (Part No. 50583B): One 100 µL vial of 10 µg/mL human AFP.

Materials Not Supplied

1. AFP Sample: serum, plasma, lysate
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Store all components at 4ºC

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-AFP Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-AFP Antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of AFP Standard in the concentration range of 10 ng/mL – 0.156 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	10 µg/mL Human AFP Standard (µL)	Assay Diluent (µL)	AFP (ng/mL)
1	4	3996	10
2	500 of Tube #1	500	5
3	500 of Tube #2	500	2.5
4	500 of Tube #3	500	1.25
5	500 of Tube #4	500	0.625
6	500 of Tube #5	500	0.313
7	500 of Tube #6	500	0.156
8	0	500	0

Table 1. Preparation of AFP Standard

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Add 100 µL of AFP sample or standard to the Anti-AFP Antibody Coated Plate. Each AFP sample, standard, blank, and control should be assayed in duplicate.
3. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
4. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Biotinylated Anti-AFP Antibody to each well.
6. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
7. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 4 above.
8. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well.
9. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
10. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 4 above. Proceed immediately to the next step.

11. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
12. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
13. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical AFP ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

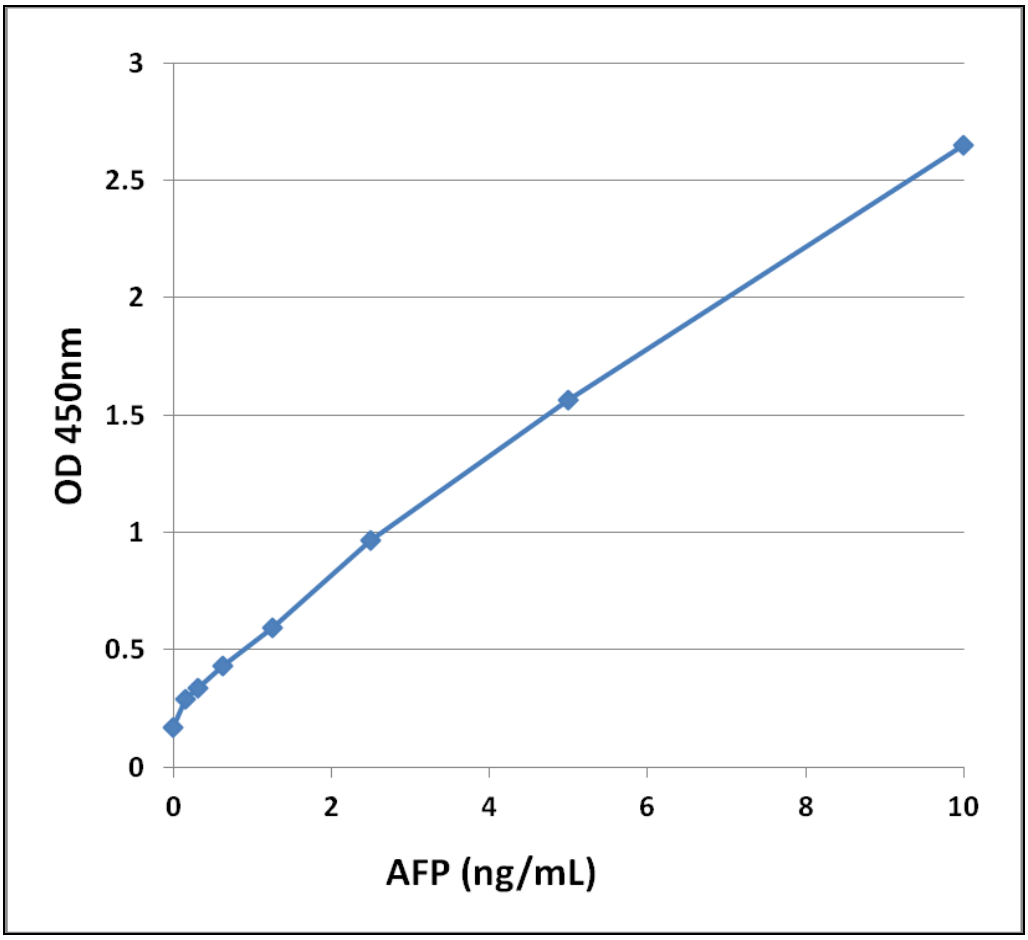

Figure 1: AFP ELISA Standard Curve

References

1. Choi, S., A. Yu, B. Kim, E. Ko, S. Park, B. Nam, J. Park (2017) *J. Gastroenterol. Hepatol.* **3**:651-658.
2. Li, J., X. Chen, M. Dai, S. Huang, J. Chen, S. Dai (2017) *Clin. Res. Hepatol. Gastroenterol.* ***17****:30045-1*.
3. Meng, W., B. Bai, Z. Bai, Y. Li, P. Yue, X. Li, L. Qiao (2016) *Discov. Med.* **118**:489-494.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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