PromedeusLab

Human Alpha fetoprotein ELISA

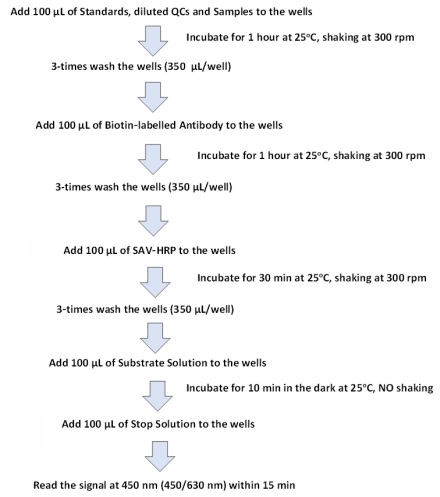
Cat. No.: PL1003

Enzyme Immunoassay for the quantitative determination of Alpha fetoprotein (AFP) in human serum and plasma.

Alpha-fetoprotein (AFP) is a 70-kDa tumor-associated fetal mammalian glycoprotein which belongs to the gene family of serum albumin. AFP is involved with both ontogenic and oncogenic growth, as well as immunoregulatory processes.[1] AFP is synthesized in the yolk sac, fetal liver, and gastrointestinal tract during pregnancy but is re-expressed in multiple adult tumours of mixed mesodermal/endodermal origin.[2] Native AFP during pregnancy as well as tumour AFP stimulates cell and tissue growth, thus tAFP supports cancerogenesis. Serum levels of AFP are elevated in patients with liver cancer as well as other types of cancer (breast, esophagus, cervical, pancreatic, endometrial, gastric, lung, rectum), and noncancer diseases cirrhosis, nephrotic syndrome, and gastritis. On the other hand, whereas patients suffering from multiple myeloma, Wilms' tumour, and other 22 types of noncancer diseases had significantly decreased median serum AFP levels than that of healthy controls.[3]
Alpha-fetoprotein is a shuttle protein that delivers nutrients through receptor-mediated endocytosis to embryotic cells. In adults, alpha-fetoprotein can shuttle drugs into alpha-fetoprotein receptor-positive myeloid-derived suppressor, regenerating and cancer cells.[4]

PRINCIPLE OF AFP ELISA

The microtiter plate is coated with the antibody specifically binding Alpha fetoprotein. The human serum and plasma are incubated in the plate with the capture antibody.
The specimen is washed out and the specifically bound protein is incubated with biotin-labelled detection antibody. Following another washing step, Streptavidin-HRP conjugate is added into the well.
Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).
The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of AFP in the specimen. The concentration of AFP in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.



Kit Contents

Item	*Qty.*
Antibody Coated Microtiter Plate	96 wells
Biotin-labelled Antibody	13 mL
Streptavidin-HRP Conjugate	13 mL
Master Standard (lyophilized)	1 vial
Quality Control A (human serum, lyophilized)	1 vial
Quality Control B (human serum, lyophilized)	1 vial
Dilution Buffer	13 mL
Wash Buffer 15x conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

MATERIAL REQUIRED BUT NOT SUPPLIED

1. Glassware and test tubes
2. Microtiter plate washer
3. Precision pipettes (various volumes) with tips
4. Orbital shaker
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

WARNINGS AND PRECAUTIONS

1. For research use only
2. For professional laboratory use
3. The reagents with different lot numbers should not be mixed
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25°C ± 2°C).

STORAGE CONDITIONS

1. The kit must be stored at 2 – 8°C.
2. The opened components can be stored for one week at 2 – 8°C.

PREPARATION OF REAGENTS

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25°C ± 2°C.

Preparation of Standards

Reconstitute lyophilized Human AFP Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min. The concentration of human AFP in Master Standard is 20 ng/mL.

Prepare set of Standard solution as follows:

Use the Master Standard for serial dilution (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 20 ng/mL (lyophilized)	See CofA	20 ng/mL
Std2	300 µL of Std1	300 µL	10 ng/mL
Std3	300 µL of Std2	300 µL	5 ng/mL
Std4	300 µL of Std3	300 µL	2.5 ng/mL
Std5	300 µL of Std4	300 µL	1.25 ng/mL
Std6	300 µL of Std5	300 µL	0.625 ng/mL
Std7	300 µL of Std6	300 µL	0.3125 ng/mL
Blank	-	300 µL	0 ng/mL

Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls with deionized/distilled water, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min and dilute them 1:10 in Dilution Buffer, prior to use, see Preparation of samples.

Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15x conc. to 700 mL of deionized/ distilled water (dH_2O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

Preparation of samples

Human EDTA plasma may be used with this assay. For long-term storage the samples should be frozen at minimum -70°C. Lipemic or haemolytic samples may cause false results.

Recommended dilution of samples is 1:10, i.e., for singlets 20 µL of sample + 180 µL of Dilution Buffer, for duplicates 30 µL of samples + 270 µL of Dilution Buffer, respectively.

Do not store the diluted samples.

ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 300 rpm.
3. Wash the wells 3-times with 1x Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of Biotin-labelled Antibody into each well. Incubate for **1 hours** at 25°C ±2°C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 µL of Streptavidin-HRP into each well. Incubate for **30 min** at 25°C ±2°C, shaking at 300 rpm.
7. Wash the wells as described in point 3.
8. Pipette 100 µL Substrate solution, incubate for 1**0 min**, at 25°C ±2°C. Avoid exposure to the light during this step.
9. Pipette 100 µL of STOP solution.
10. Read the signal at 450 or 450/630 nm within 15 min.

PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:10 as recommended and assayed. The results are multiplied by the dilution factor.

1. Sensitivity

The limit of detection, defined as a concentration of human AFP giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 0.08 ng/mL of sample.

2. Precision

Intra-assay

Sample	Mean (ng/mL)	SD	CV (%)
1	54.8	4.1	7.5
2	79.6	3.8	4.8

Inter-assay (Run – to – run)

Sample	Mean (ng/mL)	SD	CV (%)
1	5.8	0.16	2.8
2	3.6	0.02	5.4

3. Accuracy

Dilution linearity

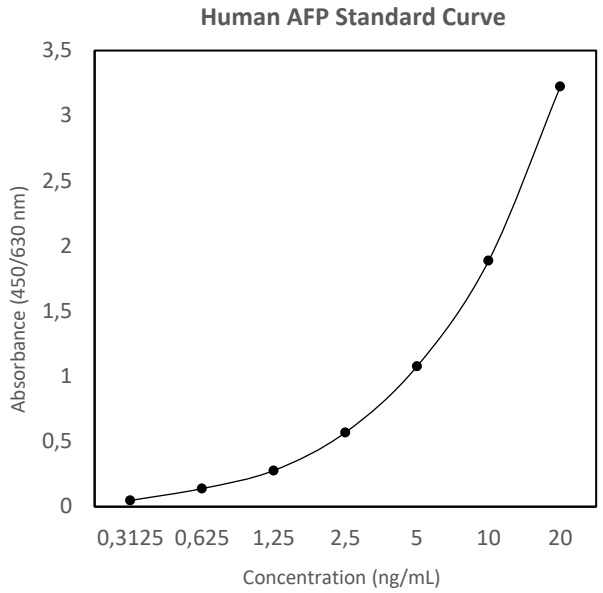
Sample	Dilution	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1		55.5	-	-
	2x	28.4	27.7	102
	4x	14.0	13.9	101
	8x	6.6	6.9	96
2		58.3	-	-
	2x	28.1	29.2	96
	4x	14.2	14.6	98
	8x	6.6	7.3	90

Spiking Recovery

Sample	Spike (ng/mL)	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1	-	6.2	-	-
	50	50.0	56.2	89
	25	27.3	31.2	88
	12.5	16.5	18.7	88

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.



RESOURCES

[1] Adigun OO, Yarrarapu SNS, Zubair M, Khetarpal S. Alpha Fetoprotein. 2022 Nov 23. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 28613501.

[2] Wong RJ, Ahmed A, Gish RG. Elevated alpha-fetoprotein: differential diagnosis - hepatocellular carcinoma and other disorders. Clin Liver Dis. 2015 May;19(2):309-23. doi: 10.1016/j.cld.2015.01.005. Epub 2015 Feb 27. PMID: 25921665.

[3] He Y, Lu H, Zhang L. Serum AFP levels in patients suffering from 47 different types of cancers and noncancer diseases. Prog Mol Biol Transl Sci. 2019;162:199-212. doi: 10.1016/bs.pmbts.2019.01.001. Epub 2019 Mar 6. PMID: 30905450.

[4] Pak VN. The use of alpha-fetoprotein for the treatment of autoimmune diseases and cancer. Ther Deliv. 2018 Jan;9(1):37-46. doi: 10.4155/tde-2017-0073. PMID: 29216804.