Tribioscience

Fast Human Alpha Fetoprotein ELISA Kit (AFP); Catalog Number: TBS3212

For the quantitative determination of human AFP concentrations in cell culture supernates, serum, and plasma.

INTRODUCTION

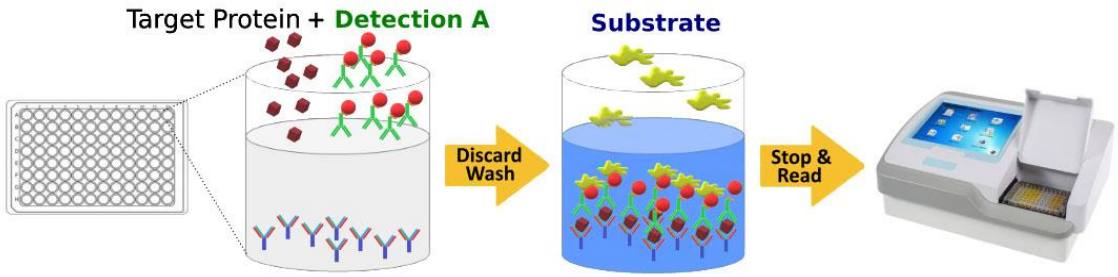
α-Fetoprotein (AFP), a member of the albuminoid superfamily (Albumin, Vitamin D-binding protein, and α-Albumin), is a fetal/tumor associated protein well known as a marker for certain cancers and congenital defects. AFP is a widely used marker indicative of chromosomal or neural tube abnormality. Low maternal serum AFP levels have been associated with a higher incidence of Down's syndrome, whereas higher levels are associated with spina bifida and anencephaly. Certain pathological conditions can trigger AFP production postnatally. Elevated AFP levels coincide with several cancers including hepatoblastoma, hepatocellular carcinoma, germ cell tumors, and certain gastric cancers. In addition, AFP can be elevated in benign hepatocellular diseases including active hepatitis and cirrhosis.

Tribioscience's Fast Human AFP ELISA is designed to quantitatively detect human AFP levels in serum, plasma, and other biological samples. The main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The hands-on time can be within 2 hours, with no need for 4-5 hours (Fig. 1). The detection range is from 10 to 100000 pg/mL. The levels of human AFP samples are parallel to the standard curves obtained using the kit standards linearly. Therefore, the kit can be used to determine relative mass values for natural human AFP protein.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique (See Fig. 1). A monoclonal antibody specific for human AFP was pre-coated onto a microplate. Standards or samples and Detection Antibody are pipetted into the wells, and concurrently incubated for 2 hours. Then, just aspirate each well, and simply wash. Following a wash to remove any unbound antibodies and samples, an **ultra-sensitive TMB substrate solution** is added to the wells for color development. The color intensity is in proportion to the amount of bound in the initial step. The intensity of the color is measured by plate reader at 450 nm.

Fig.1



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE CONDITIONS
Human AFP Microplate	TBS3212A	96 well strip microplate (12 strips of 8 wells) coated with a human AFP monoclonal antibody.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8°C.
Human AFP Standard	TBS3212B	50 µL of Recombinant human AFP protein (ng/mL).	Aliquot and store at -20°C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3212C	2.2 mL of HRP-Human AFP antibody.	May be stored for up to 3 months at 2-8°C.*
Assay Diluent	TBS3212D	15 mL of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 mL of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 mL of 2 N sulfuric acid.	

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

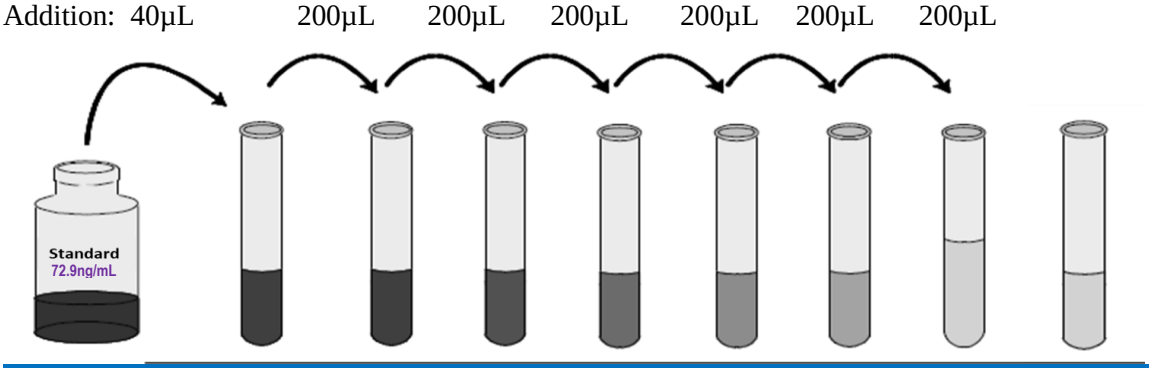
Bring all reagents to room temperature before use.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved).*

Human AFP Standard Preparation: Label test tubes as #1 through #8. Pipet 360 µL of 1x Assay Diluent into tube #1, and 200 µL into tubes #2 to #8 **as diagram below.**

1. Add 40 µL of the Human AFP Standard stock solution (72.9 ng/mL) to tube #1 and mix.
2. Make 3x serial dilutions of the standard using the Tube #1 (7290 pg/mL standard solution) from Tube #2 through #7 with sequential transfer of 200 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 7290, 2430, 810, 270, 90, 30, and 10 pg/mL. Tube #8 is Standard 8 (0 pg/mL)

Fig.2 Diagram for Human AFP standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	360	400	400	400	400	400	400	400
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	40	200	200	200	200	200	200	0
Final Conc (pg/mL)	7290	2430	810	270	90	30	10	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 2 hours with shaking**.
3. Aspirate each well, and wash for 3 times by filling each well with 200 µL Wash Buffer (*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.
4. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10 – 20 minutes** (*Protect from light*). The color becomes blue.
5. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
6. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength

correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) 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 human 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.

TYPICAL DATA

This standard curve(R^2=0.9987) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

SENSITIVITY

The minimum detectable dose (MOD) of human is typically 10 pg/ml.
The Intra-assay CV and the Inter-assay CV are <10%.

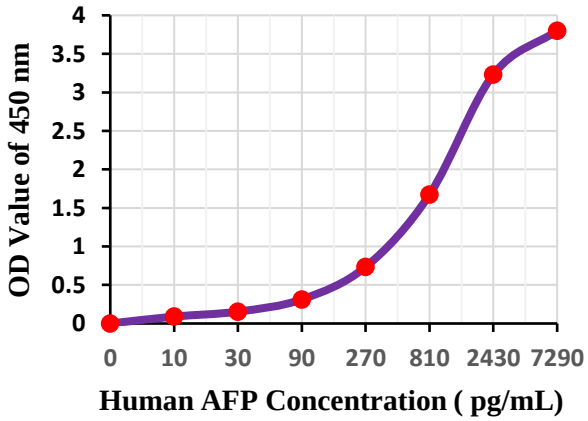
SPECIFICITY

This assay recognizes natural and recombinant human AFP.
No cross-reactivity with others.

RELATIVE PRODUCTS

Human p-Tau-181 ELISA (TBS3294)
Human Total Tau ELISA (TBS3295)
Human p-Tau-231 ELISA (TBS3296)
Human AD7 Human AD7C NTP (TBS3297)
Human Amyloid ß40 ELISA (TBS3298)
Human Amyloid ß42 ELISA (TBS3299)
Human Total Amyloid Amyloid ß ELISA (TBS32104)
Human NF-L ELISA (TBS32101)
Human IL-2 ELISA (TBS3220)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-10 ELISA (TBS3226)
Human IL-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IL-22 ELISA (TBS3229)
Human IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)
Human TNF-α ELISA (TBS3235)
Protein Assay Kit (TBS2005)
TMB Substrate System (TBS5021)

Fig.3: Human AFP Standard Curve



For research use only. Not for use in diagnostic procedures.