



Alpha-Fetoprotein (AFP) ELISA

08/16

(Catalog # K4242-100, 100 assays, Store at 4°C)

I. Introduction:

Binds copper, nickel, and fatty acids as well as, and bilirubin less well than, serum albumin. Only a small percentage (less than 2%) of the human AFP shows estrogen-binding properties. It is a common screening test during pregnancy while high level of AFP suggest the fetus may have neural tube defect. BioVision's Alpha-fetoprotein ELISA kit is a sandwich ELISA assay for the quantitative measurement of human Alpha-fetoprotein in serum, plasma and other biological fluid. The density of color is proportional to the amount of human Alpha-fetoprotein captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Alpha-fetoprotein.

III. Specificity:

Human

IV. Sample Type:

Serum, plasma and other biological fluid

V. Kit Contents:

Components	K4242-100	Part No.
Micro ELISA Plate	8 X 12 strips	K4242-100-1
AFP Standards	0.5 ml X 6	K4242-100-2
Anti-AFP Enzyme Conjugate	12 ml	K4242-100-3
Anti-AFP-Biotin Reagent	12 ml	K4242-100-4
TMB Substrate	12 ml	K4242-100-5
Wash Concentrate (20X)	25 ml	K4242-100-6
Stop Solution	12 ml	K4242-100-7

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VII. Storage and Handling:

The entire kit may be stored at 4°C. Keep Standard <-20°C, -80°C for long term storage.

VIII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment.

Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. Prepare **1X Wash buffer** by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).
2. **Sample Preparation:** Collect blood specimens and separate the serum immediately. Typically, specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens.

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.

It is recommended that all standards and samples be run at least in duplicate.

A standard curve must be run with each assay.

1. Place the desired number of coated strips into the holder.
2. Pipette 25 µl of **AFP standards** and samples.
3. Add 100 µl of **Anti-AFP-Biotin Reagent** to all wells and mix for 20-30 seconds.
4. Cover the plate and incubate for 30 minutes at room temperature (20-25°C).
5. Remove liquid from all wells. Wash wells three times 300 µl with 1X **wash buffer**. Blot on absorbent paper towels.
6. Add 100 µl of the **Anti-AFP- Enzyme conjugate** to all wells. Cover and incubate for 30 minutes.
7. Remove liquid from all wells. Wash wells three times 300 µl with 1X **wash buffer**. Blot on absorbent paper towels.
8. Add 100 µl of **TMB substrate** to all wells. Incubate for 15 minutes at room temperature.
9. Add 50 µl of **stop solution** to all wells. Shake the plate gently to mix the solution.
10. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.



X. CALCULATION:

For calculation, **(the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well)**. The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human Salbutamol concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

	OD 450 nm	Conc. ng/mL
Std 1	0.020	0
Std 2	0.072	5
Std 3	0.281	25
Std 4	0.462	50
Std 5	1.878	250
Std 6	2.447	500

XI. RELATED PRODUCTS:

- Chromogranin A (CgA) ELISA Kit (Cat. No. K4242-100)
- CA19-9 (human) ELISA Kit (Cat. No. K7427-100)
- Cancer Antigen 125 (CA-125) (human) ELISA Kit (Cat. No. K4803-100)
- Cancer Antigen 15-3 (CA15-3) (human) ELISA Kit (Cat. No. K4804-100)
- Prostate Specific Antigen (Free, human) ELISA Kit (Cat. No. K7432-100)