

DRG[®] AFP (Human Serum) (High Range) (EIA-4331)



Revised 29 June 2011 rm (Vers. 5.1)



This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE

For determination of the Alpha-Fetoprotein (AFP) in human serum.

Store at 2 to 8°C.

PRINCIPLE OF THE TEST

The AFP ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a rabbit anti-AFP antibody directed against intact AFP for solid phase immobilization (on the microtiter wells). A monoclonal anti-AFP antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react first with the immobilized rabbit antibody for 30 minutes. The wells are washed to remove any unbound antigen. The monoclonal-HRP conjugate is then reacted with the immobilized antigen for 30 minutes at room temperature resulting in the AFP molecules being sandwiched between the solid phase and enzyme-linked antibodies. The wells are washed with water to remove unbound labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of AFP is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the kits

- Rabbit anti-AFP coated microtiter plate with 96 wells.
- Zero Buffer, 13 ml.
- Reference standard set, contains 0, 20, 50, 150, 500 and 1,000 ng/ml AFP (WHO, 72/225), 0.5 ml each, ready-to-use.
- Enzyme Conjugate Reagent, 13 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided

- Distilled water.
- Precision pipettes: 10 µl, 100 µl and 1.0 ml.
- Disposable pipette tips.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph Paper
- Microtiter plate reader.



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RUO in the USA

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

All reagents should be brought to room temperature (18-25°C) before use.

ASSAY PROCEDURE

- 1. Secure the desired number of coated wells in the holder.
- 2. Dispense 10µl of standard, specimens, and controls into appropriate wells.
- 3. Dispense 100µl of Zero Buffer into each well.
- 4. Thoroughly mix for 30 seconds. It is very important to have complete mixing in this setup.
- 5. Incubate at room temperature (18-25°C) for 30 minutes.
- 6. Remove the incubation mixture by flicking plate content into a waste container.
- 7. Rinse and flick the microtiter wells 5 times with distilled or deionized water.
- 8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
- 9. Dispense 100µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
- 10. Incubate at room temperature for 30 minutes.
- 11. Remove the incubation mixture by flicking plate contents into a waste container.
- 12. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
- 13. Strike the wells sharply onto absorbent paper to remove residual water droplets.
- 14. Dispense 100µl TMB Reagent into each well. Gentle mix for 10 seconds.
- 15. Incubate at room temperature for 20 minutes.
- 16. Stop the reaction by adding 100µl of Stop Solution to each well.
- 17. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
- 18. Read optical density at 450nm with a microtiter reader within 15 minutes.

CALCULATION OF RESULTS

- 1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
- 2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
- 3. Using the mean absorbance value for each sample, determine the corresponding concentration of AFP in ng/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against AFP concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.





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AFP (ng/ml)	Absorbance (450nm)
0	0.045
20	0.273
50	0.563
150	1.339
500	2.395
1000	2.849



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