

# Alpha-feto-protein (AFP) ELISA Test Kit

## NAME AND INTENDED USE

Detection Kit for Alpha-feto-protein (AFP) (Enzyme-Linked ImmunoSorbent Assay , ELISA) .It is used in quantitative tests for Alpha-feto-protein (AFP) in human serum.

## SUMMARY AND CLINICAL SIGNIFICANCE

Alpha-Feto Protein (AFP) is a serum alpha globulin with a molecular weight 68000 daltons. AFP is normally proceeding fetal development by the yolk sac and fetal liver. Much elevation of serum AFP above normal value occurs in primary liver cancer and fetal malformation. Although AFP amounts in amniotic fluid is lower 100 times than one in fetal blood. Thereby measurement AFP amounts in amniotic fluid and pregnant women blood could diagnose whether the fetus is normal or abnormal. In addition, elevated serum AFP amounts have been measured in patients with hepatitis.

## PRINCIPLE

AFP uses a "sandwich principle", Enzyme-linked immunological sorbent assay. To measure AFP levels in serum, plastic wells coated with a monoclonal antibodies of AFP are supplied in the kit. The patient's specimen is added and, during incubation, AFP, if present, is fixed to the solid phase antibody, when another mono-antibody labelled with HRP is added, creating a HRP-antibody—AFP—antibody "sandwich". After the added of TMB substrate, the result is obtained by EIA plate reader.

## PRECAUTION FOR USERS

1. Handling should preclude any pipetting by mouth. 2. Use only pipettes with disposable tips for each specimen. 3. Do not mix materials from different master lots. Do not use kit components beyond the expiration date. All materials should be brought to room temperature before use.

## SPECIMEN COLLECTION AND PREPARATION

Serum specimens can be tested by the AFP procedure. Remove serum from the clot as soon as possible to avoid hemolysis. Covered specimens may be stored for up to 48 hours at 2-8°C. Specimens held for a longer time can be frozen at -20°C and avoid repeated freeze thaw.

Serum samples with concentrations expected to be greater than 400IU/ml should be diluted with specimen diluent.

NOTE: If need, remove by centrifugation the suspended fibrin particles or aggregates which are liable to produce falsely positive results.

## REAGENTS SUPPLIED

1. Coated Microplate: 1 plate (8×12 wells), Ready to use. Coated with anti-AFP antibody and sealed in an aluminum bag. Remove the strips in the resealable bag with a desiccant to protect from moisture after opened. Store at 2-8°C until expiration date.
2. HRP Conjugate: 1 vial of 7ml, Ready to use. Store at 2-8°C until expiration date.
3. Calibrator: 7 vials of 1ml, Ready to use. Labeled with S0 to S6 and their concentration of AFP is 0, 5, 10, 20, 50, 100, 200IU/ml. Store at 2-8°C until expiration date.
4. Control: 2 vials of 1ml, Ready to use. Store at 2-8°C until expiration date. The concentration of low value is 10.5-19.5 IU/ml. The concentration of high value is 46.3-86.0 IU/ml. Store at 2-8°C until expiration date
5. Chromogen A: 1 vial of 7ml, Ready to use. Store at 2-8°C until expiration date.
6. Chromogen B: 1 vial of 7ml, Ready to use. Store at 2-8°C until expiration date.
7. Stop Solution: 1 vial of 7ml, Ready to use. Store at 2-8°C until expiration date.
8. Wash buffer: 1 vial of 15ml, Concentrate 20-fold, diluting with deionized water before the assay. Store at 2-8°C until expiration date.
9. Plate sealer: 2 pieces. 10. Plastic resealable bag: 1 set. 11. Instruction manual: 1 copy.

## RELATED TIPS

1. This kit is designated for In-Vitro Diagnostic Use Only.
2. Wash procedure. Incomplete washing will adversely affect the test results. Wash each well 5 times with about 0.3ml distilled water. If no automatic washer is available, washing can be performed manually as follows: Invert the plate vigorously to get all water out and block the rim of well on absorbent paper for a few seconds. Filling each well with water and remain 20 seconds. Repeat these steps 5 times. Blot dry the plate by inverting the plate onto absorbent tissue, and striking a hard surface several times.
3. Drip procedure. Mix the bottle gently before use. Violent surge may cause too much foam. Invert the bottle and squeeze one or two drop on absorbent tissue to make sure there is no foam. Take the bottle upright the well and make sure the drop does not touch the rim of wells.
4. Read procedure. Using the blank well to correct the zero point of reader if single wavelength reader is used. If double wavelength readers with 450nm and 630nm are used, there is no need to correct the zero point.
5. Storage. The whole kit should be stored at 2-8°C for one year. Microplate should be taken to room temperature before opened. This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. After removing the required number of strips, the plate should be put in the plastic resealable bag with desiccants to minimize exposure to damp air.

## PREPARATIONS

1. Allow all specimens and reagents to reach room temperature and mix thoroughly by gentle inversion before use.
2. Prepare Wash Solution by diluting Wash Concentrate 20-fold with deionized water. The diluted wash solution is stable in room temperature for at least one week.

## ASSAY PROCEDURE:

1. Mark the microtitration strips to be used. Set six wells for calibrators, two wells for controls and other wells for samples.
2. Dispense 50 ul of calibrators as well as controls or samples into respective wells.
3. Dispense 50 ul of HRP Conjugate to each well.
4. Covered the strips with a plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 30 minutes.
5. Wash each well for 5 times, 20 seconds each time. (See wash procedure).
6. Dispense 50µl of chromogen A to each well.
7. Dispense 50µl of chromogen B to each well.
8. Covered the strips with a fresh plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at room temperature for 8-15 minutes.
9. Dispense 50µl of stop solution to each well and mix completely.
10. Read the absorbance of the plate within 10 minutes. (See read procedure)

## CALCULATION OF RESULTS

Computer: Use the linear fitting function, the logarithm of each calibrator concentration (Log), as X, take the logarithm of the corresponding absorbance value (Log(OD)) as Y, choose double logarithm (or full Logarithmic) Log-Log fitting the concentration of the serum to be tested is calculated from the fitted line.

Equation:  $\log OD = B * \log [\text{concentration}] + A$

## PERFORMANCE CHARACTERISTICS

1. Expected value The cut-off value of AFP concentration for healthy subjects is below 20IU/ml.
2. Sensitivity  
The detection limit of the assay is approximately 3.0 IU/ml.

## 3. Precision

Interassay ≤15%

Intraassay ≤10%

4. Specificity: No cross reactions with HSA, IgG, CEA, Ferr, PSA.

5. High-Dose-Hook Effect

No hook effect was observed in this test up to 6400IU/ml.

CODIGO: RSET111-3