**PRINCIPLE OF THE TEST**

This kit uses solid phase, double antibody sandwich principle ELISA method in which polystyrene microwell strips are pre-coated with anti-AFP antibodies. Patient’s serum or plasma sample is added and during the first incubation, the AFP if present in sample, will be captured on the wells surface. The microwells are than washed to remove unbound serum proteins. A second anti-AFP antibody conjugated to Horseradish Peroxidase (HRP-Conjugate) is added and during the second incubation, this antibody will bind to the captured AFP. The microwells are than washed to remove unbound conjugate, and Chromogen solutions are added to the wells. In wells containing the antigen-antibody-antigen (HRP) “sandwich” immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of AFP captured in the wells and to the sample respectively.

**INTENDED USE**

This AFP ELISA Kit is an enzyme linked immunosorbent assay (ELISA) for in vitro quantitative determination of α-fetoprotein (AFP) concentrations in the range of 2-400 ng/ml in human serum or plasma samples.

**MATERIALS REQUIRED BUT NOT SUPPLIED**

1. Validated microplate reader.
2. Eppendorf Tubes for dilution for samples and standards.
3. Deionized or distilled water.
4. Validated adjustable micropipettes, single and multi-channel.
5. Automatic microtiter plate washer or manual vacuum aspiration equipment.
6. 37°C incubator.

**STORAGE**

**Unopened Kit:** Store at 2 - 8°C. Do not use past kit expiration date.

**Opened/Reconstituted Reagents:** TMB Solution A; TMB Solution B; TMB Stop Solution; Wash Buffer; HRP-conjugate antibody

The above mentioned reagents should be stored for up to 1 month at 2 - 8°C.

**Microplate Wells:** Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8°C.

**PRECAUTIONS**

1. The kit should be equilibrated to room temperature (20-23°C) before opening any vials and starting the assay. It is highly recommended that the solutions be used as soon as possible after rehydration.
2. When mixing or reconstituting protein solutions, always avoid foaming.
3. Do not mix or substitute reagents with those from other lots or sources.
4. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
5. Crystals could appear in the 20X wash solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
7. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

**Alpha-Fetoprotein, AFP ELISA Kit (Quantitative)**

Prod. No.: DEIA080
Pkg. Size: 96T

**REAGENTS AND MATERIALS PROVIDED**

**Microplate:** 96 well polystyrene microplates (12 strips of 8 wells) coated with antibodies reactive to AFP (anti-AFP);

**Calibration Curve Standards:** 0ng/ml, 5ng/ml, 10ng/ml,20ng/ml,50ng/ml,100ng/ml and 200ng/ml, 2x7vials;

**HRP-Conjugate Antigen:** 12 ml, 1 vial;

**TMB Solution A:** 7 ml, 1 vial;

**TMB Solution B:** 7 ml, 1 vial;

**TMB Stop Solution:** 7 ml, 1 vial;

**Wash Buffer (20×):** 50 ml, 1 vial;

**Microtiter plate sealers:** 1 sheets;

**Plastic Sealable Bag:** 1 unit.
**SPECMEN TREATMENT**

Centrifuge the serum, plasma or cell culture supernatant samples for 10 minutes at 1,000×g. Remove particulates and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

**ELISA PROTOCOL**

1. Allow the reagents and samples to reach room temperature for at least 15-30 minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the stock Wash Buffer 1 to 20 with distilled or deionized water. Use only clean vessels to dilute the buffer.

2. Set the strips needed in strip-holder and number sufficient number of wells including seven calibration curve standards wells (e.g. B1-H1; A2-G2) and one Blank (e.g. A1, neither samples nor HRP-Conjugate antibody should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test. Run the standards in duplicates.

3. Add 50 µl calibration curve standards and 50 µl specimen into their respective wells. Note: Use a separate disposal pipette tip for each specimen as to avoid cross-contamination. Cover the plate with the plate cover and incubate for 30 minutes at 37°C. At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash buffer. Each time, allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the plate down onto blotting paper or clean towel, and tap it to remove any remainders.

4. Add 50 µl HRP-Conjugate reagent to each well except the Blank. Cover the plate with the plate cover and incubate for 30 minutes at 37°C. At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash buffer. Each time, allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the plate down onto blotting paper or clean towel, and tap it to remove any remainders.

5. Dispense 50 µl of TMB Solution A and 50 µl TMB Solution B solution into each well including the Blank, and mix by tapping the plate gently. Incubate the plate at 37°C for 15 minutes avoiding light. The enzymatic reaction between the TMB solutions and the HRP-Conjugate antibody produces blue color in calibration curve standards (except in the 0ng/ml standard well) wells and in AFP positive sample wells.

6. Using a multichannel pipette or manually, add 50 µl Stop Solution into each well and mix gently. The blue color will turn yellow after stopping the reaction.

7. Calibrate the plate reader with the Blank well and read the absorbance at 450 nm. If a dual filter instrument is used, set the reference wavelength at 630 nm. Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 5 minutes after stopping the reaction)

**INTERPRETATION OF RESULTS AND QUALITY CONTROL**

If the result reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

1. Record the absorbance (OD) obtained from the print report of the microplate reader.

2. Plot the absorbance (log-OD) for each duplicate calibration standard on the Y (logarithmic ordinate) versus the corresponding anti-HBs concentration (log-mIU/ml) on the X (logarithmic abscissa) on double-logarithmic paper (do not average the duplicates of the calibration standards before plotting).

3. Draw the standard curve through the plotted points (best-fit).

4. To determine the concentration of anti-HBs for an unknown, locate the absorbance (OD) for each unknown on the Y-axis of the graph, find the intersecting point on the standard curve, and read the concentration (log-ng/ml) from the X-axis of the graph. Calculate the concentration of the unknown in ng/ml.

**Quality control range:**

The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

1) The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450nm.
2) The OD value of 0mIU/ml standard must be less than 0.100 at 450/630nm or at 450nm after blanking.
3) The OD value of the 160mIU/ml standard must be higher than 1.500 at 450/630nm or at 450nm after blanking.
**LIMITATIONS**

1. The prevalence of the marker will affect the assay’s predictive values.
2. Non-repeatable reactive results may be obtained with any ELISA test due to the general characteristics of this type of assays. Any positive result must be interpreted in conjunction with the patient clinical information and other laboratory results.
3. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.
4. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
5. Common sources for mistakes: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
6. Samples tested using assay from different manufacturer can give similar quantitative results but some samples can give discrepancies due to the antibodies diversity and the immunological properties of AFP tested in the assay.
7. Samples with AFP concentrations above 400ng/ml may be diluted and retested. The sample’s concentration is calculated by multiplying the result by the dilution factor.

**INDICATIONS OF INSTABILITY OR DETERIORATION OF THE REAGENTS**

1. Values of the Positive or Negative controls, which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones, or contact our technical support for further assistance.
2. If after mixing of the TMB Solution A and B into the wells, the color of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

**REFERENCES**

2. Henry J.B., Clinical Diagnosis and Management by Laboratory Methods,
5. Di Bisceglie AM, Carithers RL, Gores GJ. Hepatocellular carcinoma.