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Product Information

AFP (Alpha Fetoprotein) ELISA Kit

Catalog Number: EA101028
Storage Temperature: 2 – 8°C

Instruction for Use

THIS KIT IS INTENDED FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

1 INTRODUCTION

1.1 Intended Use

The **ORIGENE AFP ELISA** is an enzyme immunoassay for measurement of alpha fetoprotein (AFP) in serum.

2 PRINCIPLE OF THE TEST

The ORIGENE AFP ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal [mouse] antibody directed towards a unique antigenic site on an AFP molecule. An aliquot of specimen sample containing endogenous AFP is incubated in the coated well with enzyme conjugate, which is an anti-AFP antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off. The amount of bound peroxidase is proportional to the concentration of AFP in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of AFP in the specimen sample.

3 PRECAUTIONS

- This kit is intended for Research Use Only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.

- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
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4 KIT COMPONENTS

4.1 Contents of the Kit

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with anti-AFP antibody (monoclonal).
2. ***Standard (Standard 0-4)***, 5 vials (lyophilized), 0.5 mL;
   Concentrations: 0 - 10 - 40 - 80 - 160 IU/mL
   Conversion: 1IU/mL = 1,21ng/mL
   The standards are calibrated against NIBSC 1st International Standard for Alphafoetoprotein AFP (AFP 1st IRP 72/225)
   See „Preparation of Reagents“;
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservatives.
3. ***Enzyme Conjugate***, 1 vial, 11 mL, ready to use,
   Anti-AFP antibody conjugated to horseradish peroxidase;
   * contains 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservatives.
4. ***Substrate Solution***, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).
5. ***Stop Solution***, 1 vial, 14 mL, ready to use,
   contains 0.5M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.

* BND = 5-bromo-5-nitro-1,3-dioxane
MIT = 2-methyl-2H-isothiazol-3-one

Note: Additional *Standard 0* for sample dilution is available upon request.

4.1.1 Equipment and material required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm) (e.g. the ORIGENE Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Bidistilled water

4.2 Storage and stability of the Kit

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for six weeks if stored as described above.

4.3 Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

Standards

Reconstitute the lyophilized contents of the standard vial with 0.5 mL bidistilled water!
Note: *The reconstituted standards are stable for 2 months at 2-8°C. For longer storage freeze at -20°C.*

4.4 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

4.5 Damaged Test Kits

In case of any severe damage of the test kit or components, ORIGENE have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN

Serum should be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
NOTE: Samples containing sodium azide should not be used in the assay.

NOTE: If an amniocentesis is necessary the specimen collection has to done before the puncture. After the amniotic puncture increased AFP values are determined.

5.1 Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Samples containing anticoagulant may require increased clotting time.

5.2 Specimen Storage

Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying.
Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) dilution 1:10: 10 µL Serum + 90 µL *Standard 0* (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

6 TEST PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Assay Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **25 µL** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3. Dispense **100 µL** ***Enzyme Conjugate*** into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **30 minutes** at room temperature.
5. Briskly shake out the contents of the wells.
   Rinse the wells 5 times with distilled water (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **100 µL** of ***Substrate Solution*** to each well.
7. Incubate for **10 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **50 µL** of ***Stop Solution*** to each well.
9. Determine the absorbance (OD) of each well at **450±10 nm** with a microtiter plate reader.
   It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and specimen samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value 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 from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 IU/mL)	0.07
Standard 1 (10 IU/mL)	0.21
Standard 2 (40 IU/mL)	0.69
Standard 3 (80 IU/mL)	1.29
Standard 4 (160 IU/mL)	1.97

7 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or ORIGENE directly.

8 LEGAL ASPECTS

8.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact ORIGENE.

8.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

9 REFERENCES

1. Ruoslahti, E. and Seppala, M., Studies of Carcino-Fetal Proteins: Physical and Chemical Properties of Human Alpha-Fetoprotein. Int. J. Cancer 7:218, 1971.
2. Gitlin, D., Perricelli, A. and Gitlin, G. M., Synthesis of Alpha-Fetoprotein by Liver, Yolk Sac, and Gastrointestinal Tract of the Human Conceptus. Cancer Res. 32:979, 1972.
3. Masseyeff, R., Gilli, G., Krebs, B., Bonet, C. and Zrihen, H., Evolution en Fonction de l'Age du Taux Serique Physiologique de l'Alpha-Foetoproteine chez l'Homme et le Rat. (French) in Alpha-Feto-Protein (Masseyeff, R., ed.) p.313. INSERM, Paris, 1974.
4. Silver, H. K. B., Gold, P., Feder, S., Freedman, So. O. and Shuster, J., Radioimmunoassay for human alpha-fetoprotein. Proc. Nat. Acad. Sci. U.S.A. 70:526, 1973.
5. Waldmann, T. A. and McIntire, K. R., The use of a radioimmunoassay for alpha-fetoprotein in the diagnosis of malignancy. Cancer 34:1510, 1974.
6. Kohn, J., Orr, A.H. McElwain, T.J., Bentall, M. and Peckham, M. J., Serum alpha-fetoprotein in patients with testicular tumors. Lancet 2:433, 1976.
7. Abelev, G.I., Alpha-fetoprotein in ontogenesis and its association with malignant tumors. Adv. Cancer Res. 14: 295, 1971.
8. Scardino, P. T., Cox, H. D., Waldermann, T. A., McIntire, K. R., Mittemeyer, B. and Javadpour, N., The value of the serum tumor markers in the staging and prognosis of germ cell tumors of the testis. J. Urol. 118:994, 1977.
9. Bosl, G.J., Lange, P. H., Fraley, E. E., Goldman, A., Nochomovitz, L. E., Rosai, J., et al., Human chorionic gonadotropin and alpha-fetoprotein in the staging of nonseminomatous testicular cancer. Cancer 47:328, 1981
10. Lange, P.H., McIntire, K.R. and Waldmann, T. A., Hakala, T.R. and Fraley, E. E., Serum alpha-fetoprotein and human chorionic gonadotropin in the diagnosis and management of nonseminomatous germ-cell testicular cancer. Medical Intelligence 259:1237, 1976.
11. Javadpour, N., McIntre, K. R. and Waldmann, T. A., Human chorionic gonadotropin (HCG) and alpha-fetoprotein (AFP) in sera and tumor cells of patients with testicular seminoma. Cancer 42:2768, 1978.
12. Waldmann, T.A. and McIntire, K. R., Serum Alpha-Fetoprotein Levels in Patients with Ataxia-Telangiectasia. Lancet 2:1112, 1972.
13. Belanger, L., Tyrosinemie Hereditaire et Alpha-1-Fetoproteine. II. Recherche Tissulaire Comparee de l'Alpha-Foetoproteine dans Deux Cas de Tyrosineimie Hereditaire. Considerations sur l'Ontogenese de la Foetoproteine Humain (French). Pathol. Bio.21:457, 1973.
14. Kew, M. C., Purves, L.R. and Bersohn, I., Serum Alpha-Fetoprotein Levels in Acute Viral Hepatitis. Gut 14:939, 1973.
15. Endo, Y., Kanai, K., Oda, T., Mitamura, K., lino, S. and Suzuki, H., Clinical Significance of alpha-Fetoprotein in Hepatitis and Liver Cirrhosis. Ann. N. Y. Acad. Sci. 259:234,1975.
16. Purves, L. R. and Purves M., Serum Alpha-Fetoprotein. VI. The Radioimmunoassay Evidence for the Presence of AFP in the Serum of Normal People and During Pregnancy. S. Afr. Med. J. 46:1290, 1972.
17. Sepalla, M. and Ruoslahti, E., Alpha-Fetoprotein: Physiology and Pathology During Pregnancy and Application to Antenatal Diagnosis. J. Perinat. Med. 1:104, 1973.
18. Engvall, E., Methods in Enzymology, Volume 70, Van Vunakis, H. and Langone, J.J. (eds.) Academic Press, New York, NY, 419 (1980).
19. Uotila, M., Ruoslahti, E. and Engvall, E., J. Immunol. Methods, 42, 11 (1981).