AFP-ELISA

KAPD1468

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LOT : 110225/1

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IN VITRO DIAGNOSTIC USE

en

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1 INTRODUCTION

1.1 Intended Use

The **DIAsource AFP ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of alpha fetoprotein (AFP) in serum. This kit is not intended to be used for the risk evaluation of trisomy 21.

1.2 Summary and Explanation

Alpha-fetoprotein (AFP) is a glycoprotein with a molecular weight of approximately 70 KD(1). AFP is normally produced during fetal and neonatal development by the liver, yolk sac, and in small concentrations by the gastrointestinal tract (2). After birth, serum AFP concentrations decrease rapidly, and by the second year of life and thereafter only trace amounts are normally detected in serum (3).
Elevation of serum AFP to abnormally high values occurs in several malignant diseases (4-7), most notably nonseminomatous testicular cancer and primary hepatocelluar carcinoma. In the case of nonseminomatous testicular cancer, a direct relationship has been observed between the incidence of elevated AFP levels and the stage of disease (8-9). Elevated AFP levels have also been observed in patients diagnosed with seminoma with nonseminomatous elements, but not in patients with pure seminoma (6,8,10-11).
In addition, elevated serum AFP concentrations have been measured in patients with other noncancerous diseases, including ataxia telangiectasia, hereditary tyrosinemia, neonatal hyperbilirubinemia, acute viral hepatitis, chronic active hepatitis and cirrhosis (12-15). Elevated serum AFP concentrations are also observed in pregnant women (16-17). Therefore, AFP measurements are not recommended for use as a screening procedure to detect the presence of cancer in the general population.

2 PRINCIPLE OF THE TEST

The DIAsource 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 patient 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 patient sample.

3 PRECAUTIONS

- This kit is for in vitro diagnostic 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.

4 KIT COMPONENTS

4.1 Contents of the Kit

1.  12x8 (break apart) strips, 96 wells;
Wells coated with anti-AFP antibody (monoclonal).

2. CAL | N
N=1 to 4, 4 vials (lyophilized), 0.5 mL;
See exact values on the vial label
Conversion: 1IU/mL = 1,21ng/mL
The calibrators 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 a preservative.

3. CAL | 0
Zero Calibrator, 1 vial (lyophilized), 0.5 ml
* contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as a preservative.
See „Preparation of Reagents"

4. Ab | HRP
(Enzyme conjugate) 1 vial, 11 mL, ready to use,
Anti-AFP antibody conjugated to horseradish peroxidase;
contains 0.03% Proclin 300 as a preservative.

5. CHROM | TMB
(Substrate solution) 1 vial, 14 mL, ready to use,
Tetramethylbenzidine (TMB).

6. STOP | SOLN
(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 *Calibrator 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)
- 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.

Calibrators
Reconstitute the lyophilized contents of the calibrator vial with 0.5 mL bidistilled water!
Note: *The reconstituted calibrators 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.

4.5 Damaged Test Kits

In case of any severe damage of the test kit or components, DIAsource ImmunoAssays S.A. 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. Patients receiving anticoagulant therapy 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 calibrator, the specimens can be diluted with *Calibrator 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 *Calibrator 0* (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Calibrator 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 calibrator, 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 calibration curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **25 µL** of each ***Calibrator, 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 calibrators, controls and patient samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each calibrator 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 calibration 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 calibration curve. Samples with concentrations higher than that of the highest calibrator 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 Calibration curve

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

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

7 EXPECTED VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted using the DIAsource AFP ELISA the following values are observed:

7.1 Normal healthy adults, non-pregnant

The lower limit of AFP concentration in normal serum is less than 1 IU/mL; the upper limit is about 10 IU/mL.

7.2 Values during pregnancy

Weeks of pregnancy	AFP [IU/mL]
10	9 - 24
11	10 - 27
12	10 - 30
13	10 - 34
14	11 - 45
15	14 - 60
16	16 - 69
17	17 - 78
18	22 - 93

Weeks of pregnancy	AFP [IU/mL]
19	32 - 103
20	42 - 121
21	48 - 139
22 - 24	56 - 224
25 - 27	95 - 357
28 - 30	135 - 435
31 - 33	141 - 423
34 - 36	121 - 380
37 - 40	93 - 321

CLINICAL IMPORTANCE

1. Maternal serum containing AFP above 2.5 times the normal median for weeks 16 to 18 of pregnancy was detected in 88% of cases of anencephaly and in 79% of cases of open spina bifida.
2. The concentration of AFP in hepatocellular carcinoma and germ cell tumor varies from the normal range up to several million IU/ml. After surgical resection, the serum AFP may drop to normal range or somewhat above it.
3. AFP may occur in serum of patients with diseases other than hepatocarcinoma or embryonal carcinoma of the testes, such as neonatal hepatitis and nonhepatic neoplasms.

8 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. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
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.

9 ASSAY CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 1.78 – 160 IU/mL.

9.2 Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

Protein		Produced Color Intensity Equivalent to AFP in serum (IU/mL)
HSA	20 mg/ml	2
Prolaktin	200 ng/ml	2
hCG	2000 ng/ml	2
SP- 1	2000 ng/ml	2
hPL	2011 µg/ml	2

9.3 Analytical Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of *Calibrator 0* and was found to be 1.78 IU/mL.

9.4 Precision

9.4.1 Intra Assay Variation

The within assay variability is shown below:

Sample	n	Mean (IU/mL)	CV (%)
1	20	25.63	3.82
2	20	105.78	5.39
3	20	77.63	3.50

9.4.2 Inter Assay Variation

The inter assay variability (between run is shown below:

Sample	n	Mean (IU/mL)	CV (%)
1	16	25.31	3.64
2	16	109.34	6.54
3	16	84.10	6.74

9.5 Recovery

Recovery of the DIAsource ELISA was determined by adding increasing amounts of the analyte to three sera of pregnant women. The percentage recoveries were determined by comparing expected and measured values of the samples.

		Sample 1	Sample 2	Sample 3
Concentration [IU/mL]		30.86	115.20	69.02
Average Recovery		92.9	94.0	99.1
Range of Recovery [%]	from	86.7	93.4	92.6
	to	99.5	94.7	106.5

9.6 Linearity

Three samples (serum) containing different amounts of analyt were serially diluted (up to 1:16) with zero calibrator and assayed with the DIAsource ELISA. The percentage recovery was calculated by comparing the expected and measured values for the analyt.

		Sample 1	Sample 2	Sample 3
Concentration [IU/mL]		39.7	75.6	128.4
Average Recovery		102.0	95.5	96.8
Range of Recovery [%]	from	90.9	86.2	92.9
	to	115.0	109.3	101.3

10 LIMITATIONS OF USE

10.1 Interfering Substances

Any improper handling of samples or modification of this test might influence the results.
Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of AFP in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 1600 IU/mL of AFP.

11 LEGAL ASPECTS

11.1 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 DIAsource.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 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.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. 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.

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	Used symbols
	Consult instructions for use
	Storage temperature
	Use by
LOT	Batch code
REF	Catalogue number
CONTROL	Control
I V D	In vitro diagnostic medical device
	Manufacturer
	Contains sufficient for <n> tests
WASH SOLN CONC	Wash solution concentrated
CAL 0	Zero calibrator
CAL N	Calibrator #
CONTROL N	Control #
Ag 125I	Tracer
Ab 125I	Tracer
Ag 125I CONC	Tracer concentrated
Ab 125I CONC	Tracer concentrated
	Tubes
INC BUF	Incubation buffer
ACETONITRILE	Acetonitrile
SERUM	Serum
DIL SPE	Specimen diluent
DIL BUF	Dilution buffer
ANTISERUM	Antiserum
IMMUNOADSORBENT	Immunoadsorbent
DIL CAL	Calibrator diluent
REC SOLN	Reconstitution solution
PEG	Polyethylene glycol
EXTR SOLN	Extraction solution
ELU SOLN	Elution solution
GEL	Bond Elut Silica cartridges
PRE SOLN	Pre-treatment solution
NEUTR SOLN	Neutralization solution
TRACEUR BUF	Tracer buffer
	Microtiterplate
Ab HRP	HRP Conjugate
Ag HRP	HRP Conjugate
Ab HRP CONC	HRP Conjugate concentrate
Ag HRP CONC	HRP Conjugate concentrate
CONJ BUF	Conjugate buffer
CHROM TMB CONC	Chromogenic TMB concentrate
CHROM TMB	Chromogenic TMB solution
SUB BUF	Substrate buffer
STOP SOLN	Stop solution
INC SER	Incubation serum
BUF	Buffer
Ab AP	AP Conjugate
SUB PNPP	Substrate PNPP
BIOT CONJ CONC	Biotin conjugate concentrate
AVID HRP CONC	Avidine HRP concentrate
ASS BUF	Assay buffer
Ab BIOT	Biotin conjugate
Ab	Specific Antibody
SAV HRP CONC	Streptavidin HRP concentrate
NSB	Non-specific binding
2nd Ab	2nd Antibody
ACID BUF	Acidification Buffer
DIST	Distributor