

abia AFP



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IVD



Note: Changes highlighted ★



abia

Intended use

Abia AFP is an enzyme immunoassay for the quantitative determination of alpha-fetoprotein (AFP) concentration in human serum.

For professional use only.

Clinical value

Alpha-fetoprotein (AFP) is a glycoprotein with a molecular weight of approximately 70 kDa. AFP is normally produced during fetal and neonatal development by the liver, yolk-sac and in small concentrations by the gastrointestinal tract. After birth, serum AFP concentrations decrease rapidly, and by the second year of life and thereafter only trace amounts are normally detected in serum (less than 10 IU/ml).

Elevation of serum AFP to abnormally high values occurs in several malignant diseases, most notably nonseminomatous testicular cancer and primary hepatocellular 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. Elevated AFP levels have also been observed in patients diagnosed with seminoma with nonseminomatous elements, but not in patients with pure seminoma.

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. Elevated serum AFP concentrations are also observed in pregnant women. Therefore, AFP measurements are not recommended for use as a screening procedure to detect the presence of cancer in the general population.

Principle of the test

Abia AFP is a one-step immunoassay, based on the principle of the “sandwich” method.

The assay system utilizes high affinity and specificity monoclonal antibodies (enzyme conjugated and immobilized) directed against a distinct antigenic determinant on the intact AFP molecule.

The test sample is allowed to react simultaneously with the two antibodies, resulting in the AFP molecules being sandwiched between the solid phase and enzyme-linked antibodies.

The unbound components are removed by washing. After addition of the solution containing TMB and hydrogen peroxide, the wells with bound conjugate develop a blue color which is converted to yellow after the reaction has been stopped with sulphuric acid.

The color intensity is directly proportional to the concentration of AFP in the specimen and can be read at 450 nm.

Kit contents

	S	
AFP Ab coated plate	1	polystyrene plate 12 × breakable 8-well strips coated with monoclonal anti-AFP Ab
Conjugate	1 × 12 ml	ready to use; HRP-labeled monoclonal anti-AFP Ab; transparent or slightly opalescent pink liquid
Calibrator 0	1 × 2.0 ml	protein based buffer not containing AFP; pale yellow liquid
Calibrator 1	1 × 0.5 ml	protein based buffer containing AFP in concentration approx. 5 IU/ml; pale yellow liquid
Calibrator 2	1 × 0.5 ml	protein based buffer containing AFP in concentration approx. 20 IU/ml; pale yellow liquid
Calibrator 3	1 × 0.5 ml	protein based buffer containing AFP in concentration approx. 50 IU/ml; pale yellow liquid
Calibrator 4	1 × 0.5 ml	protein based buffer containing AFP in concentration approx. 150 IU/ml; pale yellow liquid
Calibrator 5	1 × 0.5 ml	protein based buffer containing AFP in concentration approx. 300 IU/ml; pale yellow liquid
Control serum	1 × 0.5 ml	protein based control containing AFP; colorless or pale yellow
Washing solution (concentrated 25-fold)	1 × 50 ml	phosphate saline buffer; colorless or pale yellow liquid
TMB/substrate solution	1 × 12 ml	ready to use; citric acid buffer containing TMB and H ₂ O ₂ ; colorless liquid
Stopping reagent 0.2M H ₂ SO ₄	1 × 25 ml	ready to use; 0.20 mol/l sulphuric acid solution; colorless liquid
Protective film	1	
Plastic dish	2	
Plastic zip-lock bag	1	

The calibrators were calibrated using a WHO 1st IRP 72/225. Exact concentration levels for calibrators and control serum are given on the labels on a lot specific basis. For conventional units: IU/ml x 1.205 = ng/ml.

All components are stable until expiration date of the kit when stored at 2–8 °C in a tightly sealed package. Expiration date is indicated on the package. Once opened, the components should be used within 2 months. Concentration of preserving agents: <=0.1 %.

Materials and equipment required but not provided

- purified water
- automatic or semiautomatic, adjustable or preset pipettes or multipipettes
- disposable pipette tips
- automatic microplate washer
- microplate reader equipped with 450 nm filter

Safety notes

- as no known test method can offer complete assurance that infectious agents are absent, reagents and samples should be handled as if capable of transmitting infectious disease; any equipment directly in contact with samples and reagents should be considered as contaminated
- do not eat, drink, smoke or apply cosmetics in the laboratory
- do not pipette by mouth
- avoid any contact of the reagents and samples with the skin and mucosa; wear lab coats and disposable gloves when handling them; thoroughly wash your hands after work
- avoid spilling samples or solutions containing samples. Wipe spills immediately and decontaminate affected surfaces
- all materials contacted with specimens or reagents, including liquid and solid waste, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations

Precautions

- do not use reagents without label or with damaged label/package
- do not use expired reagents
- do not change the assay procedure; perform all subsequent steps without interruption
- do not mix reagents from different lots
- do not mix the caps of vials
- do not run the EIA test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals
- do not let the wells dry once the assay has been started
- do not use the same container and tips for different liquid components of the kit and samples
- do not reuse the coated plates
- do not reuse the removed protective film
- do not expose the reagents to excessive heat or sunlight during storage and test procedure
- do not freeze the reagents

Collection and handling of specimens

- collect blood specimens according to the current practices
- use serum for testing; performances of the test have not been evaluated on other biological fluids
- separate the clot or red cells from serum as soon as possible to avoid any haemolysis
- do not use sera preserved with sodium azide
- do not use contaminated, hyperlipaemic and hyperhaemolysed specimens
- the samples with hyperproteinaemia and hyperbilirubinaemia were not specially tested
- before testing samples with observable particulate matter should be clarified by centrifugation
- suspended fibrin particles or aggregates may yield reactive results
- do not heat the samples
- samples can be stored at 2–8 °C within 72 hours or deep-frozen at -20 °C
- no more than one freeze/thaw cycle is allowed

Procedural notes

- before use wait 30 minutes for the reagents to stabilize to room temperature (20–25 °C)
- check appearance of the reagents
- lost vacuum in the bag of the coated plate will not affect the performance of the test
- check the pipettes and other equipment for accuracy and correct operation
- the washing procedure is a critical step; for the detailed washer settings see section “Washing procedure”
- for the description of test procedure with the automated analyzers see section “Automated analyzers”

Washing procedure

Please contact your representative for protocols for recommended washers and procedures. In general the following protocol is recommended:

- flow-through washing with a volume not less than 300 µl per well is used
- repeat 5 times
- do not allow the wells to become dry during the assay procedure
- ensure that no liquid is left in the well (use double aspiration in the final step where possible)
- avoid to tap out the plate
- residual volume lower than 10 µl is not critical for following steps of the test procedure
- when using a microplate washer clean the wash head frequently to prevent contamination

Preparation of reagents

Number of strips to be used	1	2	3	4	5	6	7	8	9	10	11	12
Working washing solution: mix the reagents thoroughly by inversion Stability: 14 days at 18–24 °C or 28 days at 2–8 °C												
Washing solution (concentrated 25-fold), ml	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	40.0
Purified water, ml	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0	648.0	720.0	792.0	960.0

Test procedure

abia AFP for the quantitative determination of alpha-fetoprotein (AFP) concentration in human serum

- 1** Take the required number of coated strips. Place the unused strips back into the bag; reseal the foil-lined package in plastic zip-lock bag. Do not remove desiccant.

- 2** Reserve one or two wells for TMB/substrate solution control (blank).
Add 100 µl conjugate into each well except blank.

- 3** Analyze each calibrator, control serum, and sample duplicate.
Add 25 µl of calibrators 0 - 5 into appropriate wells.
Add 25 µl of control serum into appropriate wells.
Add 25 µl of samples to be tested in rest of the wells.
Rinse the pipette tip each time in the conjugate by pressing the plunger button down 2-3 times before ejecting the tip. The total time should not exceed 10 min.
Mix the contents of the wells for 30 seconds by careful tapping on the edge of the plate, then cover the plate with protective film.

- 4** Incubate for 120 minutes at room temperature 20-25 °C.

- 5** Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant).
Add not less than 300 µl of working washing solution into each well and aspirate. Perform this procedure 5 times. Use double aspiration in the final step where possible.

- 6** Add 100 µl of TMB/substrate solution to all the wells. Keep the plates in a dark place for 30 minutes at 20-25 °C.

- 7** Add 150 µl of stopping reagent into each well. Mix gently for 5-10 sec.

- 8** Read the optical density at 450 nm using a plate reader within 20 minutes after stopping reaction.

Automated analyzers

Validated protocols for automated analyzers can be obtained from your representative. For the instrumentation without established validated protocol follow section “Test procedure” and ensure all requirements described in section “Precautions” are followed. All protocols for automated analyzers must be fully validated prior usage.

Calculation and interpretation of the results

Assay validation

Results of an assay are valid if the following criteria for the controls are met.

The absorbance (OD) of blank value should be not more than 0.100 at 450 nm.
The absorbance (OD) of calibrator 5 (approx. 300 IU/ml) should be greater than 1.300.
Calculated value of control serum should be within established range.

Calculation procedure

- 1 Calculate the mean optical density of each calibrator duplicate.
- 2 Calculate the mean optical density of each sample duplicate.
- 3 Subtract the mean absorbance value of the “blank” from the mean absorbance values of the calibrators, control and serum samples.
- 4 Draw a calibration curve on linear graph paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis.
- 5 Read the values of the samples directly off the calibration curve.
If immunoassay software is being used, a 4-parameter curve is recommended.

If a sample reads more than 300 IU/ml then dilute it with calibrator 0. The result obtained should be multiplied by the dilution factor.

Example	OD 1	OD 2	Mean OD - blank (here 0.042)	Value, IU/ml
Calibrator 0	0.041	0.042	0.000	0.00
Calibrator 1	0.094	0.091	0.051	5.00
Calibrator 2	0.274	0.268	0.230	20.00
Calibrator 3	0.647	0.625	0.595	50.00
Calibrator 4	1.773	1.733	1.712	150.00
Calibrator 5	2.613	2.675	2.603	300.00
Sample	0.515	0.510	0.471	40.00

This data is for illustration only and should **not be used** to calculate samples. Each user should obtain his or her own data and standard curve.

Performance characteristics

Analytical sensitivity

The analytical sensitivity (limit of detection) was calculated by determining the variability of the calibrator 0 based on 24 analysis runs additional 2 x SD. Limit of detection defined at 1.00 IU/ml.

Specificity

	Concentration	Cross reactivity, %
Human serum albumin	12.50 mg/ml	< 0.0001
	25.00 mg/ml	< 0.0001
	50.00 mg/ml	< 0.0001
	100.00 mg/ml	< 0.0001

Precision

	Mean, IU/ml	SD	CV, %
Intra-assay, sample 1	145.04	6.19	4.27
Intra-assay, sample 2	41.18	1.08	2.63
Inter-assay, sample 1	145.08	9.39	6.47
Inter-assay, sample 2	40.08	2.43	6.07

Accuracy

The assay was compared with an commercially available enzyme immunoassay as a reference test. The total number of specimens was 267. The values ranged from 1 to 174 IU/ml. The least square regression equation and the correlation coefficient were computed for abia AFP in comparison with the reference method.

The least square regression analysis was $y = 1.084(x) + 1.83$ with correlation coefficient 0.9032.

Expected normal value

	Range, IU/ml	
Adult population	0.00	10.00

Normal value ranges may vary slightly among different laboratories. It is strongly recommended that each laboratory should determine its own range of expected normal values.

Limitations of test

- the assay was validated only for the determination of AFP in human serum
- only calibrator 0 may be used to dilute any high serum samples. The use of any other reagent may lead to false results
- the results obtained with this assay should never be used as the sole basis for clinical diagnosis. Any laboratory result is only a part of the total clinical picture of the patient
- the assay contains reagents to minimize interference of HAMA and heterophilic antibodies. However, extremely high titers of HAMA or heterophilic antibodies may interfere with the test results
- no hook effect was observed up to a AFP concentration of 2 000 IU/ml
- not intended for newborn screening

References

1. Bates S.E. Clinical applications of serum tumor markers. *Ann Intern Med* 1991; 115:623-8.
2. Wu J.C., Lee S.D., Hsiao K.J., et al. Mass screening of primary hepatocellular carcinoma by alpha-fetoprotein in a rural area of Taiwan-a dried blood spot method. *Liver* 1988; 8:100-4.
3. Lee H.-S., Chung Y.H., Kim C.Y. Specificities of serum alpha-fetoprotein in HBsAg+ and HBsAg patients in the diagnosis of hepatocellular carcinoma. *Hepatology* 1991; 14:68-72.
4. Di Bisceglie A.M., Rustgi V.K., Hoofnagle J.H., Dusheiko G.M., Lotze M.T. Hepatocellular carcinoma. *Ann Intern Med* 1988;108:390-401.
5. Sato Y, Nakata K, Kato Y, et al. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. *N Engl J Med* 1993; 328:1802-6.
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Key to symbols used



Manufacturer



For in vitro diagnostic use



Catalogue number



Batch code



Expiry date



Storage temperature limitation



Do not use if package is damaged



Do not reuse



Sufficient for [n] tests



Consult Instructions for use



Caution, consult documents



Changes highlighted

Hazard and precautionary statements for certain kit components

Stopping reagent



Warning

H315

Causes skin irritation.

H319

Causes serious eye irritation.

P264

Wash hands thoroughly after handling.

P280

Wear protective gloves/protective clothing/eye protection/face protection.

P302 + P352

IF ON SKIN: Wash with plenty of soap and water.

P305 + P351 +
P338

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Conjugate, calibrators 0 - 5, control serum



Warning

H317

May cause an allergic skin reaction.

P261

Avoid breathing dust/fume/gas/mist/vapours/spray.

P280

Wear protective gloves/protective clothing/eye protection/face protection.

P302 + P352

IF ON SKIN: Wash with plenty of soap and water.

P333 + P313

If skin irritation or rash occurs: Get medical advice/attention.

Attention!

For complete precautionary statements and detailed information see safety data sheets (SDS).



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