Alfa-Fetoprotein(AFP) ELISA

REF:1317 001 96 test

Intended Use

Microplate based ELISA (enzyme linked immunosorbent assay) for the quantitative determination of Alpha-fetoprotein(AFP) in non hemolyzed human serum.

Background

AFP is a glycoprotein with a molecular mass of about 70000Da. During the ontogenesis AFP is produced mostly in yolk-sac and embryonal liver and, to less extent, in gastrointestinal tract.AFP level in serum of pregnant women continuously increases and reaches the maximum (up to 400U/mL) at the middle of the third trimester. Serum AFP measurement in pregnant women is an important method for early diagnostics of some inborn diseases. Besides, this method is widely used in obstetrics for diagnostics of multiple pregnancy, prenatal death and threatening abortion.AFP measurement can be also used for the diagnostics and monitoring of different forms of cancer. For instance, high AFP level is often connected with primary hepatomas, testis teratomas and ovary tumors.

Assay Principle

This assay is a “sandwich” type of solid-phase enzyme immunoassay, based on two monoclonal antibodies that are specific for different epitopes of AFP molecules. One of these antibodies is conjugated with horseradish peroxidase (HRP); the other is coated onto the inner surface of microwells. The AFP molecules from the serum sample bound to both immobilized antibody and anti-AFP-peroxidase conjugate.Then the wells are washed with wash solution to remove any material not bound to their inner surface. Quantity of the bound conjugate is directly proportional to AFP level in the sample. A substrate (TMB solution) is added resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance. The color intensity is proportional to the amount of AFP in the sample. AFP concentration in the patient sample is read from a standard curve that is processed in each assay

Reagents

Microplate (MP)

12 breakable 8 wells plate (Total 96 wells) coated with anti-AFP monoclonal antibodies

Control

Protein-based solution containing known AFP concentration. Exact concentrations are printed on the vial label

Sample diluent

Enzyme Conjugate

Solution containing anti-AFP monoclonal antibodies conjugated with HRP

AFP calibrators

Protein-based solutions containing known AFP concentrations. Exact concentrations are printed on the vial label.

Substrate (TMB solution): 3,3’,5,5’-tetramethyl benzidine solution in citrate buffer containing hydrogen peroxide

Wash Solution Concentrate

Wash solution P, 20X concentrated:surfactant in buffered saline

Stop Solution

1 N HCl solution

Materials Required but not Provided

1- Automatic pipettes 2- Incubator
3- Equipment for rinsing wells.4- Elisa reader 5- Vortex tube mixer.
6- Disinfectant 7- Adsorbent material

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Temperature Limitation
IVD	For in-vitro diagnostic use		Use by/Expiration Date
LOT	Batch Code/Lot number		CAUTION. Consult instructions for use
REF	Catalogue Number		
	Consult instructions for use		Manufactured by

Reagents Storage and Stability

1- Avoid exposure to direct sunlight during incubations;
2- Kit is stored at 2-8°C and stable till the expirtation date. After initial opening the kit is stable till expiration date if stored at 2-8 °C.
3- Wash solution prepared for use: at room temperature for 5 days

Reagent Preparation

1. Bring all reagents to room temperature (18-25 ^{O}C) prior to use for at least 30 minutes.
2. Liquid calibrators and controls are ready to use.
3. Add 1 volume of Wash Solution Concentrate to 19 volumes of distilled water and mix well. Prepare the required volume according to the number of assayed samples.
   For example: Add 5ml of wash solution to 95ml of distilled water.
4- Protect substrate from direct light.

Specimen Collection and Preservation

1- Serum only is suitable for analysis. The only acceptable anticoagulants are heparin, EDTA and sodium citrate. Specimen should be promptly separated from cells after blood collection.

2- Cap and store the samples at 18-25 ^{O}C for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ^{O}C up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ^{O}C. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortexing or by inverting 10 times. After thawing, bring to room temperature and mix well by gently shaking.

Precautions and warnings

1. Despite being confirmed as negative to all viruses, It is recommended that all human sourced controls be considered potentially infectious.

2. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.

3. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.

4. Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.

5. Wash the wells completely. Each well must be fully injected with wash solution. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, dry the liquids in each well. Strike the microplate onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate strip washer.

6. Do not touch or splash the rim of the well with conjugate.

7. Do not mix or use components from kits with different batch codes.

8. When manual pipette is used, complete pipetting of all controls, samples within 10 minutes.

9. It is important that the time of reaction in each well is held constant to achieve reproducible results.

10. The addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and Stop Solution should be added in the same sequence to eliminate any time deviation during reaction.

11. When handling conjugate vials, change gloves that have contacted human plasma/sera since introduction of human IgG/IgM will result in a neutralized conjugate.

Assay Procedure

1. Pipette 100 µL of conjugate into each well, except wells A1-A2 (blank).
2. Pipette 20 µL of controls,calibrators or samples in duplicates and shake for 1-2 mins at room temperature.
Leave wells A1-A2 empty for blank!
Note: total time of dispensing must not exceed 15 minutes, otherwise the test result may be unreliable, because the time of incubation will substantially vary for different samples.
3-Incubate for 60 minutes at +37 °C
4. Wash wells 5 times
5. Pipette 100 µL of substrate into each well (including blank)
6. Incubate strips at room temperature for 20 minutes in the dark
7. Pipette 100 µl of stop solution into each wel(including blank) in the same sequence and speed as used for dispensing substrate and shake for 1-2 min at room temperature.
11.Read the absorbance at 450 nm within 20 min

Assay Scheme	
Conjugate	100 µl
Calibrators,controls or sample	20 µl
Incubation time	60 minutes
Washing step	5 times
Substrate	100 µl
Incubation time in dark	20 minutes
Stop solution	100 µl
Read absorbance	450nm

Wash procedure

1-Remove the contents of the wells into container with disinfectant;
2-Dispense 300 µL of wash solution, shake the plate carefully for 5–10 sec and remove the contents of the wells; repeat 5 times;
3-Strike the wells sharply on absorbent material to remove any liquid residue.

Expected Values:

Normal adults: 0-14IU/ml (0-18ng/ml)

For pregnant women (IU/ml):

week 14: 13-47
week 15: 17-70
week 16: 19-62
week 17: 22-69
week 18: 25-60
week 19: 28-92
week 20: 29-104
Conversion factor : 1IU/ml = 0.8ng/ml

Quantitative determination

Specialized software for quantitative determination is recommended. Mean Absorbance of calibrators are plotted versus their respective AFP concentrations using 4PL or 5PL fit (see typical standard curve, fig. 1).Calculate concentration of AFP in samples using standard curve.
Any extrapolation of the standard curve to AFP above the nominal value of the calibrator 5 (approximately 300 IU/mL) is forbidden. In this case the sample should be diluted 50- or 2000-fold with sample diluent and re-tested. Multiply the measured concentration of pre-diluted samples by corresponding dilution factor.

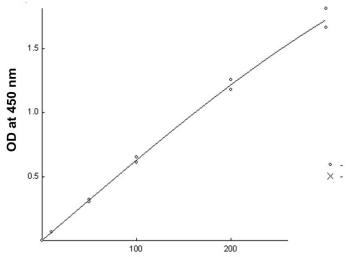

Fig.1. Example of typical standard curve.
Do not use for evaluation of real assay data!
AFP concentration, IU/mL

Limitations

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. For diagnosis establishment, a physician is supposed to consider all available clinical and laboratory findings

Analytcal Sensitivity

Analytical sensitivity of AFP kit i.e.concentration, that can be distinguished from zero calibrator, is 0.9IU/mL. It was defined as mean absorbance of 10 replicates of calibrator 0 plus two standard deviations.

Specificity

No cross-reaction was detected between anti-AFP monoclonal antibodies used in the assay and serum albumin, chorionic gonadotropin, human placental lactogen and human immunoglobulin.

Performance Charateristics

Measurement pricision

Within run (Repeatability)

With one human serum based panel, using one batch of reagent in replicates of 100 samples data are as following:

Mean = 12.4 **SD =** 0.079 **CV =** 3.6%

Run to run (Reproducibility)

With one human serum based panel, using one batch of reagent in replicates of 100 samples across 3 separate runs data are as following:

Mean =13.4 **SD =** 0.048 **CV =** 7.43 %

References

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