PATHOZYME® ALPHA-FETOPROTEIN Ref OD307

Enzyme-Immunoassay (EIA) for the quantitative determination of AFP in human serum

Store at 2°C to 8°C. DO NOT FREEZE

For in-vitro diagnostic use only.

INTRODUCTION

Alpha fetoprotein is produced in the foetus by the liver and the yolk sac. After birth the serum levels of AFP decrease to barely detectable levels within the first year of life. An increase in serum AFP levels in non-pregnant individuals can be an indicator of hepatic or testicular cancer. In pregnancy the maternal serum AFP levels are increased due the production of AFP by the foetus.

AFP is a glycoprotein with a molecular weight of 70000 Da. In all cases of metastatic hepatic carcinoma the AFP serum levels are increased. In 50% of nonseminomatous testicular cancer the AFP levels will also be elevated. Other cancers such as pancreas, stomach, colon and lung might also elevate the AFP levels. In a small number of non-malignant conditions (5-10%) such as hepatitis and cirrhosis of the liver the serum AFP levels might also be elevated.

AFP in maternal serum has a maximum level at 30 weeks. After this point the AFP decreases rapidly, that by the 36th week the AFP level is less than 2% of the maximum level. Especially elevated AFP levels can indicate multiple gestation, foetal death, open-neural tube defects amongst other conditions. Low maternal AFP levels can indicate Down syndrome, spontaneous abortion, molar pregnancy amongst other problems.

INTENDED USE

PATHOZYME AFP is an Enzyme Immunoassay (EIA) for the quantitative determination of Alpha-Fetoprotein (AFP) in human serum.
For professional use only.

PRINCIPLE OF THE TEST

Specific rabbit anti-AFP antibodies are prepared, purified, and coated onto microtitration wells. Test sera are applied and incubated with Zero Buffer. If human AFP is present in the specimen, it will bind to the antibodies in the wells. Unbound material is washed away and mouse monoclonal anti-AFP antibody, labelled with Horseradish Peroxidase enzyme (Conjugate) is added. The conjugate binds to the AFP which is bound to the antibodies. Unbound material is again washed away.
On addition of the Substrate (TMB), a colour will develop only in those wells in which enzyme is present, indicating the presence of AFP. The enzyme reaction is stopped by the addition of dilute Hydrochloric Acid and the absorbance is then measured at 450nm. The concentration of AFP is directly proportional to the colour intensity of the test sample.
This test has been calibrated against in house standards. There is no International standard for this test.

CONTENTS

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	96
Microtitre Plate	**12 x 8 wells x 1**
Breakable wells coated with specific antibody contained in a resealable foil bag with a desiccant.	
Cal A 0 ng/ml	**0.5 ml**
Reference Standard: Human serum free of AFP. Ready to use. (Colourless)	
Cal B 5 ng/ml	**0.5 ml**
Reference Standard: AFP diluted in human serum. Ready to use. (Colourless)	
Cal C 20 ng/ml	**0.5 ml**
Reference Standard: AFP diluted in human serum. Ready to use. (Colourless).	
Cal D 50 ng/ml	**0.5 ml**
Reference Standard: AFP diluted in human serum. Ready to use. (Colourless).	
Cal E 150 ng/ml	**0.5 ml**
Reference Standard: AFP diluted in human serum. Ready to use. (Colourless).	
Cal F 300 ng/ml	**0.5 ml**
Reference Standard: AFP diluted in human serum. Ready to use. (Colourless).	
Conj	**17 ml**
Anti-AFP HRP Conjugate: Anti-AFP conjugated to HRP. Ready to use (Pink)	
Buf AS	**11 ml**
Zero Buffer: Phosphate based buffer containing stabilising proteins. Ready to use. (Yellow)	
Subs TMB	**11 ml**
Substrate Solution: 3,3', 5,5' Tetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)	
Soln Stop HCl 1M	**11 ml**
Stop Solution: Hydrochloric Acid diluted in purified water. Ready to use. (Colourless)	
Instruction Leaflet and EIA Data Recording Sheet	**1 + 1**

MATERIAL REQUIRED BUT NOT PROVIDED

Micropipettes: 100µl, 200µl and 1000µl
Disposable pipette tips
Absorbent paper
Microplate reader fitted with a 450nm filter
Graph paper
Thoroughly clean laboratory glassware.

PRECAUTIONS

PATHOZYME AFP contains materials of human origin which have been tested and confirmed negative for HCV, HIV I and II antibodies and HBsAg by approved procedure at single donor level. Because no test can offer complete assurance that products derived from human source will not transmit infectious agents it is recommended that the reagents within this kit be handled with due care and attention during use and disposal. All reagents should, however, be treated as potential Biohazards in use and for disposal. Do not ingest.

PATHOZYME AFP reagents do not contain dangerous substances as defined by current UK Chemicals (Hazardous Information and Packaging for Supply) regulations. All reagents should, however, be treated as potential biohazards in use and disposal. Final disposal must be in accordance with local legislation.

PATHOZYME AFP Stop Solution is diluted Hydrochloric Acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with water.

PATHOZYME AFP reagents contain 1% Proclin™ 300* as a preservative which may be toxic if ingested. In case of contact, rinse thoroughly with running water and seek medical advice.

*Proclin™ 300 is a trade mark of ROHM & HAAS Limited.

STORAGE

Reagents must be stored at temperatures between 2°C to 8°C.

Expiry date is the last day of the month on the bottle and the kit label. The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Do not use reagents after the expiry date.

Exposure of reagents to excessive temperatures should be avoided. Do not expose to direct sunlight.

DO NOT FREEZE ANY OF THE REAGENTS (except for standards storage) as this will cause irreversible damage.

SPECIMEN COLLECTION AND PREPARATION

Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required.

Do not use haemolysed, contaminated or lipaemic serum for testing as this will adversly affect the results.

Serum may be stored at 2°C to 8°C for up to 48 hours prior to testing. If longer storage is required, store at –20°C for up to 1 year. Thawed samples must be mixed prior to testing.

Do not use Sodium Azide as a preservative as this may inhibit the Peroxidase enzyme system.

Do not repeatedly freeze-thaw the specimens as this will cause false results.

REAGENT PREPARATION

All reagents should be brought to room temperature (20°C to 25°C) and mixed gently prior to use. Do not induce foaming.

LIMITATIONS OF USE

The use of samples other than serum has not been validated in this test. There is no reuse protocol for this product.
When making an interpretation of the test it is strongly advised to take all clinical data into consideration. Diagnosis should not be made solely on the findings of one clinical assay.

ASSAY PROCEDURE

1. Bring all the kit components and the test serum to room temperature (20°C to 25°C) prior to the start of the assy.
2. One set of Standards should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the standards and the test serum on the EIA Data Recording Sheet provided.
3. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.
4. Dispense 20µl of Test Serum and Standards into the assigned wells.
5. Dispense 100µl of Zero Buffer into each well and mix for 30 seconds. It is very important to mix completely.
6. Incubate the plate for 30 minutes at room temperature (20ºC to 25ºC).
7. At the end of the incubation period, discard the contents of the wells by flicking plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Ensure adequate disinfectant is contained in the Biohazard container.
8. Hand Washing: Fill the wells with a minimum of 300µl of distilled water per well. Flick plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Wash the empty wells 5 times.
9. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
10. Machine Washing: Ensure that 300µl of distilled water is dispensed per well and that an appropriate disinfectant is added to the waste collection bottle. Wash the empty wells 5 times. After washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
11. Dispense 150µl Anti-AFP HRP Conjugate into each well and mix gently for 5 seconds.
12. Incubate for 30 minutes at room temperature (20°C to 25°C).
13. Wash plate as described above.
14. Dispense 100µl of Substrate Solution into each well and gently mix for 5 seconds.
15. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
16. Stop the reaction by adding 100µl Stop Solution to each well.
17. Gently mix for 30 seconds to ensure that the blue colour changes completely to a yellow colour.
18. Read the optical density immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

TROUBLESHOOTING

For use by operatives with at least a minimum of basic laboratory training.

Do not use damaged or contaminated kit components.

Use a separate disposable tip for each sample to prevent cross contamination.

Duplication of all standards and specimens, although not required, is recommended.

Specimens and standards should be run at the same time to keep testing conditions the same.

It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used, since pipetting of all Standards and specimens should be completed within 3 minutes. A full plate of 96 wells may be used if automated pipetting is available.

Replace caps on all reagents immediately after use.

Avoid repeated pipetting from stock reagents as this is likely to cause contamination.

Do not mix reagents or antibody coated strips from different kits. When dispensing, care should be taken not to touch the surface of the well.
Do not allow reagent to run down the sides of the well. Prior to the start of the assay bring all reagents to room temperature (20°C to 25°C). Gently mix all reagents by gentle inversion or swirling.
Once an assay has been initiated, the wells should not be allowed to become dry during the assay.

Do not contaminate the Substrate Solution as this will render the whole kit inoperative.

Check the precision and accuracy of the laboratory equipment used during the procedure to ensure reproducible results.

The unused strips should be resealed in the foil bag, containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.

CALCULATION OF RESULTS

Calculate the mean absorbance value (A450) for each set of Standards and specimens. Construct a standard curve by plotting the mean absorbance from each Standard against its concentration in ng/ml on graph paper. Use the mean absorbance values for each specimen to determine the corresponding concentration of AFP in ng/ml from the standard curve.
If levels of controls or users known samples do not give expected results, test results must be considered invalid.
If using a software package choose a quadratic regression curve fit.

EXPECTED VALUES AND SENSITIVITY

The graph produced by the calibrators should be Hyperbolic in shape with the OD450 of the calibrators proportional to their concentration. The OD of calibrator A should be less than 0.75 and the OD of calibrator F greater than 1.5 for the assay results to be valid.

In high risk patients, AFP values between 100ng/ml and 350 ng/ml suggest a diagnosis of hepatocellular carcinoma and levels over 350ng/ml usually indicate the disease. Approximately 97% of healthy subjects have AFP levels less than 8.5 ng/ml. It is recommended that each laboratory establish its own normal range. The minimum detectable concentration of PATHOZYME AFP is estimated to be 2.0 ng/ml.

EVALUATION DATA

Calibrated to major competitors and in house standards.
The co-efficient of variation of **PATHOZYME AFP** is less than or equal to 10%.

In an evaluation between the Omega **Pathozyme AFP** kit and the Abbott AxSym Kit for samples with levels between 15.6 ng/ml and 330 ng/ml the following data was generated.

Number of Samples	79
Correlation Co-efficient	0.985
Slope	1.038
Intercept	0.729
Omega Mean	55.12 ng/ml
Abbott Mean	52.24 ng/ml

These kits were shown to give good correlation.

REFERENCES

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(4) **Sell, L. S.** Cancer markers of the 1990s. *Clin. Lab. Med.* 1990;10:1-37.
(5) **Hirai, H., Nishi, S., Watabe H.** et al. Some chemical, experimental and clinical investigations on alpha-fetoprotein. In: Hirai, H., Miyaji, T. (eds.). *Alpha-fetoprotein and heptatoma.* Gann. Monogr. 1973;14:19-34.

QUICK REFERENCE TEST PROCEDURE

1. Dispense 20µl of Test Serum or Standards and 100µl of Zero Buffer into each well and mix gently for 30 seconds.
2. Incubate for 30 minutes at room temperature (20°C to 25°C).
3. Discard well contents and wash five times with distilled water.
4. Dispense 150µl of Anti-AFP HRP Conjugate into each well. Gently mix for 5 seconds.
5. Incubate in for 30 minutes at room temperature (20°C to 25°C).
6. Discard well contents and wash five times.
7. Add 100µl of Substrate Solution to each well and gently shake for 5 seconds.
8. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
9. Add 100µl of Stop Solution to each well and gently shake for 30 seconds.
10. Read the Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

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