Expected values and sensitivity
In high-risk patients, AFP values between 100 and 350 ng/ml suggest a diagnosis of hepatocellular carcinoma, and levels over 350 ng/ml usually indicate the disease. Approximately 97% of the 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 AFP by this assay is estimated to be 2.0 ng/ml.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. Accuracy: In an internal study **Qualisa™ AFP** was evaluated against commercially available licensed kit with 90 random clinical samples, & **Qualisa™ AFP** has demonstrated 100% clinical correlation with the commercially available licensed kit.
   Precision: **Qualisa™ AFP** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with **Qualisa™ AFP**	Coefficient of Variation (CV)
Level 1	10	7.08	6.99
Level 2	10	52.30	7.78
Level 3	10	163.05	7.64

B) External Evaluation:
Qualisa™ AFP ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Qualisa™ AFP** ELISA has demonstrated 100% correlation with the reference method.
*Data file: Zephyr Biomedicals (A Division of Tulip Diagnostics Pvt. Ltd.).

IMPORTANT NOTE

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multiple channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of standards & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

BIBLIOGRAPHY

(1). Abelev G I. Alpha-fetoprotein as a marker of embryo-specific differentiation in normal and human tissues. **Transplant Rev** 1974;20:3-37. (2). Hirai H. Alpha fetoprotein. In: Chu T M, ed. **Biochemical markers for cancer.** New York: Marcel Dekker, 1982:23-59. (3). Chan D W, Miao Y C. Affinity chromatographic separatoin of alpha-fetoprotein variants: Development of a mini-column procedure and application to cancer patients. **Clin Chem** 1986;32:2143-2146. (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 hepatoma**. **Gann Monogr** 1973:14:19-34.

SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	Batch Number / Lot Number
Manufacturer	*In vitro* Diagnostic Medical Device	This side up	Contains sufficient for <n> tests
Use by	Catalogue Number	Do not reuse	

Manufactured by:
Zephyr Biomedicals
A Division of Tulip Diagnostics (P) Ltd.
M 46-47, Phase III B, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.
Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

0422/VER-01

Qualisa™
Microwell Enzyme Immunoassay | AFP
Alpha-Fetoprotein

Enzyme Linked Immunosorbent assay for the Quantitative Determination of Alpha-Fetoprotein (AFP) in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Qualisa™ AFP Sandwich ELISA test is intended for the quantitative determination of Alpha-fetoprotein (AFP) in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

Alpha-fetoprotein (AFP) is normally produced during fetal and neonatal development by the liver, yolksac, 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.
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 ASSAY

Qualisa™ AFP Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-AFP antibody for solid phase (microtiter wells) immobilization and another mouse anti-AFP antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the AFP antibody coated microtite wells and incubated with the Zero Buffer. If human AFP is present in the specimen, it will combine with the antibody on the well. The well is then washed to remove any residual test specimen, and AFP antibody labeled with horseradish peroxidase (conjugate) are added resulting in the AFP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation the wells are washed and bound enzyme is detected by adding substrate. The reaction is stopped after specified time with stop solution and absorbance is determined for each well using an ELISA reader. The concentration of AFP is directly proportional to the color intensity of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with anti- AFP antibody.
- AFP Enzyme Conjugate. Ready to use.
- AFP Zero buffer
- TMB Substrate. Ready to use
- Stop Solution. Ready to use
- AFP Standard set of 6 standards labeled as A to F in liquid form. Ready to use. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X).

Materials required but not provided

- Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

STORAGE AND STABILITY

1. **Qualisa™ AFP** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the dessicant has changed from blue to white at the time of opening the pouch, another coated microwells pouch should be used.
3. Diluted Wash Buffer is stable upto one week when stored at 2-8°C.

SPECIMEN COLLECTION

1. Collect Blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens and standards should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water), Mix well before use.

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **20 µl** of standards and serums into the appropriate wells.
2. Dispense **100 µl** of zero buffer into each well. Incubate at room temperature (18-25°C) for **30 mins**.
3. After incubation, empty the microtitre wells and wash the plate 5 times with 350µl of diluted wash buffer. Strike the microtitre plate sharply onto absorbent paper towel to remove all residual droplets.
4. Dispense **100 µl** of enzyme conjugate reagent into each well. Incubate at room temperature (18-25°C), for **30 minutes**.
5. After incubation, empty the microtitre wells and wash the plate 5 times with 350µl of diluted wash buffer. Strike the microtitre plate sharply onto absorbent paper towel to remove all residual droplets.
6. Dispense **100 µl** of TMB substrate into each well. Incubate at room temperature(18-25°C) in the dark, for **20 minutes**.
7. Stop the reaction by adding **100 µl** of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
8. Read the optical density at 450/630 nm with a microtiter plate reader within **15 minutes**.

Add **20** µl Standards/samples into the respective Microwells

↓

Add **100** µl Zero Buffer in each well

↓

Apply plate sealer and incubate for **30** minutes at **18-25°C**

↓

Wash Microwells **5 times** with 350 µl **of** diluted wash buffer

↓

Add **100** µl Enzyme Conjugate in each well

↓

Apply plate sealer and incubate for **30** minutes at **18-25°C**

↓

Wash Microwells **5 times** with 350 µl of diluted wash buffer

↓

Add **100** µl substrate in each well

↓

Incubate for **20** Minutes at **18-25°C in dark**

↓

Add **100** µl stop solution in each well

↓

Read results @450nm (Ref.600-700nm) within **15** Minutes

CALCULATION OF RESULTS

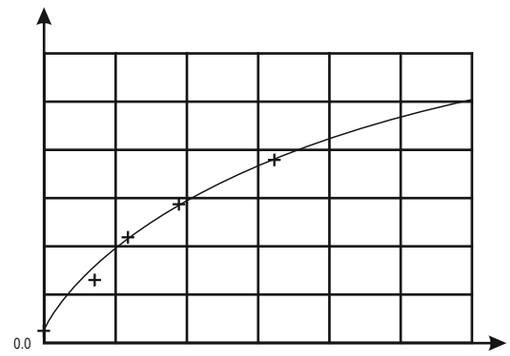
Construct a standard curve by plotting the absorbance obtained from each reference standards against its concentration in ng/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of AFP in ng/ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Standard curve

Results of a typical standard run with optical density reading at 450nm (ref 600-700nm) shown in the Y axis against AFP concentrations shown in the X axis.

Suggest: Use 4-Parameter Standard curve to calculate sample values.

AFP Values (ng/ml)	Absorbance
A	0.010
B	0.140
C	0.461
D	1.030
E	1.985
F	2.067



This standard curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her own standard curve and data.