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## ALPHA-FETOPROTEIN (AFP)

REF

Catalog No. C29-332

IVD

### For In Vitro Diagnostic Use Only

**Chemiluminescence Enzyme Immunoassay for the Quantitative Determination of ALPHA-FETOPROTEIN (AFP) in Human Serum**

#### INTRODUCTION

Chemiluminescence Immunoassay (CLIA) detection using Microplate luminometers provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies, such as Enzyme-linked immunosorbent assays (ELISA).

ELISA employs a label enzyme and a colorimetric substrate to produce an amplified signal for antigen, haptens or antibody quantitation. This technique has been well established and considered as the technology of choice for a wide variety of applications in diagnostics, research, food testing, process quality assurance and quality control, and environmental testing. The most commonly used ELISA is based on colorimetric reactions of chromogenic substrates, (such as TMB) and label enzymes.

Recently, a chemiluminescent immunoassay has been shown to be more sensitive than the conventional colorimetric method(s), and does not require long incubations or the addition of stopping reagents, as is the case in some colorimetric assays. Among various enzyme assays that employ light-emitting reactions, one of the most successful assays is the enhanced chemiluminescent immunoassay involving a horseradish peroxidase (HRP) labeled antibody or antigen and a mixture of chemiluminescent substrate, hydrogen peroxide, and enhancers.

The CLIA Kits are designed to detect glow-based chemiluminescent reactions. The kits provide a broader dynamic assay range, superior low-end sensitivity, and a faster protocol than the conventional colorimetric methods. The series of the kits covers Thyroid panels, such as T3, T4, TSH, Hormone panels, such as hCG, LH, FSH, and other panels. They can be used to replace conventional colorimetric ELISA that have been widely used in many research and diagnostic applications. Furthermore, with the methodological advantages, Chemiluminescent immunoassay will play an important part in the Diagnostic and Research areas that ELISAs can not do.

Immunospec CLIA Kits have been validated on the **MPL1** and **MPL2** microplate luminometers from Berthold Detection System, **Lus2** microplate luminometer from Anthos, **Centro LB960** microplate luminometer from Berthold Technologies, and **Platolumino** from Stratec Biomedical Systems AG. We got acceptable results with all of those luminometers.

#### INTRODUCTION OF AFP IMMUNOASSAY

Alpha-fetoprotein (AFP) is a glycoprotein with a molecular weight of approximately 70,000 daltons. 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.

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

Immunospec 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 monoclonal anti-AFP antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the AFP antibody coated microtiterwells 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. The conjugate will bind immunologically to the AFP on the well, resulting in the AFP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a incubation at room temperature, the wells are washed with wash buffer to remove unbound labeled antibodies.

A solution of chemiluminescent substrate is added and then read relative light units (RLU) in the appropriate Luminometers. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of AFP in the sample. By reference to a series of AFP standards assayed in the same way, the concentration of AFP in the unknown sample is quantified.

#### MATERIALS AND COMPONENTS

##### **Materials provided with the test kit:**

1. Antibody-coated microtiter wells. 96 wells per bag.
2. Zero buffer, 12 ml.
3. Reference standard set, contains 0, 5, 20, 50, 150, and 300 ng/ml (WHO, 72/225) AFP, liquid.
4. Enzyme Conjugate Reagent, 18 ml.
5. 20x Wash Buffer, 30 ml
6. Chemiluminescence Reagent A, 6.0 ml.
7. Chemiluminescence Reagent B, 6.0 ml.

##### **Materials required but not provided:**

- Precision pipettes: 20µl~200µl 0 ml.
- Disposable pipette tips.
- Distilled water.
- Glass tubes or flasks to mix Chemiluminescence Reagent A and Chemiluminescence Reagent B.

- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter plate Luminometer

#### SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

#### STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (One year from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

#### REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. To prepare substrate solution, make a 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
3. Prepare the washing solution by diluting 1 part of the 20X PBS concentrate to 19 parts of distilled water.

#### ASSAY PROCEDURES

1. Secure the desired number of coated wells in the holder.
2. Dispense 20µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100µl of zero buffer into each well.
4. Thoroughly mix for 10 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature (18-22°C) for 30 minutes.
6. Remove the incubation mixture by flicking plate content into a waste container.
7. Rinse and flick the microtiter wells 5 times with wash buffer.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 150µl of Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 30 minutes.
11. Remove the incubation mixture by flicking plate contents into a waste container.
12. Rinse and flick the microtiter wells 5 times with wash buffer.
13. Strike the wells sharply onto absorbent paper to remove residual water droplets.
14. Dispense 100 µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
15. Read wells with a chemiluminescence microwell reader 5 minutes later. (between 5 and 20 min. after dispensing the substrates).

#### Important Note:

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. If there are bobbles existing in the wells, the false readings will be created. Please use distilled water to remove the bobbles before adding the substrate.

#### CALCULATION OF RESULTS

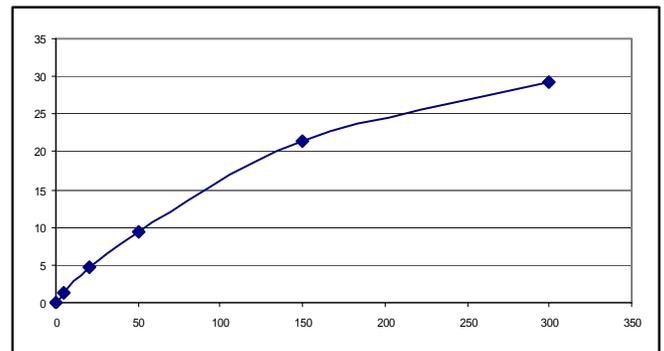
1. Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.

2. We recommend use proper software to calculate the results. The best curve fitting used in the assays are quadratic regression or 4-parameter regression. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against AFP concentration in ng/ml on linear graph paper, with RLU 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 of AFP in ng/ml from the standard curve.

#### EXAMPLE OF STANDARD CURVE

Results of a typical standard run are shown below. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is running at the same time.

AFP (ng/ml)	RLU (10 <sup>5</sup> )
0	0.1
5	1.3
20	4.6
50	9.5
150	21.5
300	29.3



#### 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.

#### LITERATURES

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fetoprotein. In: Hirai H, Miyaji T, eds. **Alpha-fetoprotein and hepatoma. Gann Monogr** 1973;14:19-34.



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