REFERENCES

SUMMARY AND EXPLANATION
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
The AFP is a direct solid phase sandwich ELISA method. The samples and diluted anti-AFP-HRP conjugate are added to the wells coated with MAb to beta subunit. AFP in the patient’s serum binds to anti-AFP MAb on the well and the anti-AFP second antibody then binds to AFP. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of AFP in the samples. A standard curve is prepared relating color intensity to the concentration of the AFP.

<table>
<thead>
<tr>
<th>MATERIALS PROVIDED</th>
<th>96 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Microwell coated with AFP MAb</td>
<td>12x8x1</td>
</tr>
<tr>
<td>2. AFP Standard: 6 vials (ready to use)</td>
<td>0.5ml</td>
</tr>
<tr>
<td>3. AFP Enzyme Conjugate: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>4. Incubation Buffer: 1 bottle</td>
<td>12ml</td>
</tr>
<tr>
<td>5. TMB Substrate: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>6. Stop Solution: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>7. 20X Wash concentrate: 1 bottle</td>
<td>25ml</td>
</tr>
</tbody>
</table>

Cat#: GWB-DEBD36 (96 Tests)
For Order and Inquiries, please contact
GenWay Biotech
6777 Nancy Ridge Drive, San Diego, CA 92121
Tel (858) 458-0866, Fax (858) 458-0833,
sales@genwaybio.com
MATERIALS NOT PROVIDED
1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY
1. Store the kit at 2-8°C.
2. Keep micro wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS
1. Potential biohazardous materials:
   The calibrator and controls contain human source components which have been tested
   and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA
   licensed reagents. However, as there is no test method that can offer complete assurance
   that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be
   handled at the Biosafety Level 2, as recommended in the Centers for Disease
   Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical
   Laboratories." 1984
2. This test kit is designed for research use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit
   reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of
   different lots should not be mixed.
5. It is recommended that serum samples be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise
   pipetting, as well as following the exact time and temperature requirements prescribed are
   essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING
1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5
   days, store frozen at (-20°C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENTS PREPARATION
Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled
or deionized water. Store at room temperature (18-26°C).

ASSAY PROCEDURE
Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.
1. Place the desired number of coated strips into the holder
2. Pipette 25 µl of AFP standards, control and patient’s sera.
3. Add 100 µl of the Incubation buffer to all wells and mix for 20-30 seconds.
4. Cover the plate and incubate for 60 minutes at room temperature (18-26°C).
5. Remove liquid from all wells. Wash wells three times with 300 µl using 1X wash buffer.
   Blot on absorbent paper towels.
6. Add 100µl of the Enzyme conjugate to all wells. Cover and incubate for 30 minutes.
7. Remove liquid from all wells, and repeat the washing process as in step 5.
8. Add 100 µl of TMB substrate to all wells.
9. Incubate for 15 minutes at room temperature.
10. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
11. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping
    solution.

CALCULATION OF RESULTS
The standard curve is constructed as follows:
1. Check AFP standard value on each standard vial. This value might vary from lot to lot.
   Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for the AFP standards (vertical axis)
   versus the AFP standard concentrations in ng/ml (horizontal axis) on a linear graph paper.
   Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the
   value for each control or unknown sample.

Example of a Standard Data

<table>
<thead>
<tr>
<th>Std</th>
<th>OD 450 nm</th>
<th>Conc. ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std 1</td>
<td>0.041</td>
<td>0</td>
</tr>
<tr>
<td>Std 2</td>
<td>0.147</td>
<td>5</td>
</tr>
<tr>
<td>Std 3</td>
<td>0.490</td>
<td>25</td>
</tr>
<tr>
<td>Std 4</td>
<td>0.735</td>
<td>50</td>
</tr>
<tr>
<td>Std 5</td>
<td>1.696</td>
<td>250</td>
</tr>
<tr>
<td>Std 6</td>
<td>2.285</td>
<td>500</td>
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EXPECTED VALUES
It is recommended that each laboratory establish its own normal ranges based on a
representative sampling of the local population. The following values for AFP may be used as
initial guideline ranges only: AFP Normal Range = Less Than 20 ng/ml

LIMITATIONS OF THE TEST
1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.