ichroma™ AFP

INTENDED USE
ichroma™ AFP is designed to measure Alpha Feto Protein (“AFP”) in a quantitative manner in human whole blood, serum and plasma based on the principle of lateral flow immunoassay.

INTRODUCTION
Alpha-fetoprotein (AFP) is a α1-globulin family of human plasma proteins and a glycoprotein with a molecular weight approximately 70 kDa. AFP is produced primarily in the liver of developing fetus. It can be found in maternal blood and in amniotic fluid since it is secreted into fetal serum. A great increase of AFP concentration in a limited number of patients diagnosed with various diseases such as gastrointestinal tract cancer, viral hepatitis, chronic active hepatitis, alcoholic cirrhosis, and adenocarcinomas of lung, pancreas, and gall bladder. Since AFP is well known to be an important prognostic indicator of non-seminomatous testicular cancer, its most definitive role is on monitoring post-treatment clinical status and the post therapeutic evaluation of patients.

PRINCIPLE
The principle of ichroma™ AFP is a sandwich immunofluorescence assay. A fluorescence conjugated Anti-AFP in a detection buffer binds to Alpha Feto Protein (AFP) in a sample to form an antigen-antibody complex. These antigen-antibody complexes are then captured by another Anti-AFP that has been immobilized on a test strip, as the sample mixture migrates through a nitrocellulose matrix. The more AFP in a sample, the more antigen-antibody complexes are accumulated on a test strip, resulting in higher signal intensities of fluorescence. ichroma™ Reader analyzes and reads the fluorescence intensity, and shows the AFP concentration in a sample.

COMPONENTS AND REAGENTS
ichroma™ AFP consists of Test Cartridges, ID Chip and Detection Buffer Tubes.
- The Test Cartridge contains a test strip on which Anti-AFP and Rabbit IgG have been immobilized in the test line and the control line, respectively.
- Each Test Cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed Test Cartridges are packed in a box which also contains the ID Chip.
- The Detection Buffer contains fluorochrome-labeled Anti-AFP, fluorescence-labeled anti-rabbit IgG, bovine serum albumin (BSA) as a stabilizer and sodium azide in PBS as a preservative.
- The Detection Buffer is dispensed in each Detection Buffer Tube. 25 Detection Buffer Tubes are packed in box.

WARNINGS AND PRECAUTIONS
- For in vitro diagnostic use only.
- Carefully follow the instructions and procedures described in this insert.
- Lot numbers of all the test components (Test cartridge, ID Chip and Detection Buffer) must match each other.
- Do not interchange the test components from different lots.
- Do not use the test components beyond the expiration date.
- The Test Cartridge should remain sealed in its original pouch until use. Do not use damaged or opened Test Cartridge.
- Allow a minimum of 30 minutes for the Test Cartridge to attain room temperature if stored in a refrigerator.
- The Detection Buffer should attain room temperature prior to testing.
- ichroma™ AFP as well as the ichroma™ Reader should be kept away from vibration exposure and/or magnetic fields. During normal usage, ichroma™ Reader may produce minor vibrations which should be regarded as normal.
- One Detection Buffer Tube should be used for processing one sample only. One Test Cartridge should also be used for testing one processed sample only. The Detection Buffer Tube as well as the Test Cartridge should be discarded after single use.
- Used Detection Buffer Tubes, pipette tips and Test Cartridge should be handled carefully and disposed of by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.

STORAGE AND STABILITY
- The Test Cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4 - 30°C.
- The Detection Buffer Dispensed in a tube is stable for 20 months if stored at 4 - 30°C.
- Once the Test Cartridge pouch is opened, the test should be performed immediately.

LIMITATIONS OF THE TEST SYSTEM
ichroma™ AFP provides accurate and reliable results subject to the following constraints:
- ichroma™ AFP should be used only in conjunction with ichroma™ Reader.
- Use freshly collected samples for testing.
- Any anticoagulants other than EDTA should be avoided.
- Samples must be kept at room temperature prior to testing. Appropriate precautions must be exercised to test samples shipped from overseas.
- The effectiveness of the test is highly dependent on storage conditions of test components and samples.
- Tests may yield false positive results due to (i) cross-reactions between some components of serum with the capture/detector antibodies and/or (ii) non-specific adhesion of certain components having similar epitopes to bind to these antibodies.
- Tests may also yield false negative results due to the nonresponsiveness of antigens to antibodies resulting from the former’s epitopes being masked by some unknown components, such that the antigens cannot be detected or captured by the antibodies. False negative results may also be obtained due to the instability or degradation of AFP antigen due to time and/or temperature making it unrecognizable by the antibodies.
- Other factors interfering with tests and causing erroneous results may include technical/procedural errors, the degradation of the test components/reagents as well as the presence of interfering substances in samples.
- Any clinical diagnosis based on the test results must be supported by comprehensive judgment of a concerned physician, clinical symptoms and any other relevant test results.

SAMPLE COLLECTION AND PROCESSING
ichroma™ AFP Tests can be performed using human whole blood, serum or plasma.
- It is recommended to test samples within 24 hours after collection.
- Separate serum and plasma from whole blood within 3 hours after collection by centrifugation.
- If tests cannot be performed within 24 hours after collection, samples should be immediately frozen below -20°C. It is not recommended, however, to keep samples in a refrigerator for more than 3 months.
- Frozen samples should be used one time only. Repeated freezing and thawing may decrease test values.

**MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>REF</th>
<th>CHROMA™ AFP -25</th>
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</thead>
</table>

**Test Cartridge Box:**
- Sealed Test Cartridges: 25
- ID Chip: 1
- Package Insert: 1

**Box containing Detection Buffer Tube**
- Detection Buffer Tubes: 25

**MATERIALS REQUIRED BUT SUPPLIED ON DEMAND**

Following items can be purchased separately from ichroma™ AFP. Please contact your sales division for more information.

- ichroma™ Reader REF FR203
- Thermal Printer
- Transfer Pipette (75 µL, 30 µL)

**TEST SETUP**

1. Check the test components of ichroma™ AFP: Sealed Test Cartridge, ID Chip and Detection Buffer Tubes.
2. Ensure that the lot number of the Test Cartridge matches that of the ID Chip as well as the Detection Buffer Tubes.
3. Keep the sealed Test Cartridge and the Detection Buffer Tubes (if previously stored in the refrigerator) at room temperature for at least 30 minutes prior to testing. Place the Test Cartridge on a clean, dust-free and flat surface.
4. Turn on ichroma™ Reader.
5. Insert the ID Chip into the ID port in ichroma™ Reader.
6. Press the “Select” button in ichroma™ Reader.
   (Please refer to the ichroma™ Reader Operation Manual for further information and operating instructions.)

*Operation condition for ichroma™ AFP*

**Temperature:** 20 – 30 °C

**Humidity:** < 70%

**TEST PROCEDURE**

1. Take out one tube of Detection Buffer from refrigerator and leave it at room temperature.
2. Draw 30 µL of whole blood (15 µL of serum, plasma or Control) with a transfer pipette and add it to the tube containing Detection Buffer.
3. Mix well the specimen with Detection buffer by tapping or inverting the tube.
4. Take 75 µL of sample mixture and load it onto the well of disposable Cartridge.
5. Leave the Cartridge at room temperature for 15 min before inserting the device into the holder.
6. To scan the sample-loaded Test Cartridge, insert it into the Test Cartridge holder in ichroma™ Reader. Ensure proper orientation of direction of the Test Cartridge and push it all the way inside the Test Cartridge holder. An arrow has been marked on the test cartridge especially for this purpose.
7. Press “Select” button in ichroma™ Reader to start the scanning process.
8. ichroma™ Reader will immediately scan the sample-loaded Test Cartridge.
9. The test results will be shown on the display screen of ichroma™ Reader.

**INTERPRETATION OF TEST RESULT**

- ichroma™ Reader automatically calculates the test results and displays the AFP concentration of samples in terms of ng/mL.
- Working range of ichroma™ AFP is 5 - 350 ng/mL and 5 ng/mL.
- ichroma™ AFP should be considered as a screening tool only. Please consult a physician to discuss the test results.

**QUALITY CONTROL**

- Quality control testing is a part of Good Laboratory Practices to confirm the expected results and the validity of the assay. Quality control testing should be performed at regular intervals.
- Prior to using a new lot, control reagents should be tested to confirm the test procedures and to verify whether the test produces expected results.
- Quality control testing should also be performed whenever there is any question concerning the validity of the test results.
- Control reagents are not provided with ichroma™ AFP. For more information, contact our Sales Team for assistance.
- ichroma™ AFP has a built-in internal control that satisfies the routine quality control requirements. This internal control test is performed automatically each time a clinical sample is tested. An invalid result from the internal control leads to displaying an error message on the ichroma™ Reader indicating that the test should be repeated.

**PERFORMANCE CHARACTERISTICS**

1. **Specificity:** Other bio-molecules, such as Hb, Bilirubin, Triglyceride, Ascorbic acid, Glucose, CEA, PSA, ALP, Troponin I, CK-MB, Albumin, and myoglobin component were added to test specimen with much higher level than their physiological level in normal blood. There was no significant interference with the AFP measurement, nor was there any significant assay cross-reactivity with other disease-related biomarkers in blood.

2. **Imprecision:** For studying intra-assay imprecision, 10 replicates of each of the three concentrations of control reagents were tested. For studying inter-assay imprecision, 10 replicates of each of the three concentrations of control reagents were tested by three different persons.

<table>
<thead>
<tr>
<th>AFP [ng/mL]</th>
<th>Intra-assay</th>
<th>Inter-assay</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>20</td>
<td>20.51</td>
<td>1.11</td>
</tr>
<tr>
<td>80</td>
<td>79.81</td>
<td>3.49</td>
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<tr>
<td>175</td>
<td>179.27</td>
<td>4.42</td>
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</tbody>
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3. **Comparability:** The AFP concentrations of 126 samples were quantified by using ichroma™ AFP and AsSYM (Abbott) as per the prescribed test procedures. The test results were compared and their compatibility was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y=0.9843X+0.0868 and R=0.995, respectively.
REFERENCES

Note: Please refer to the table below to identify various symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>Read instructions for use</td>
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<tr>
<td>Use by</td>
<td></td>
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<tr>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td>REF</td>
<td>Catalog number</td>
</tr>
<tr>
<td>Caution</td>
<td></td>
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<tr>
<td>Manufacturer</td>
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<td>Authorized representative of the European Community</td>
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<tr>
<td>IVD</td>
<td>In vitro diagnostic medical device</td>
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<tr>
<td>Temperature limit</td>
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<tr>
<td>Do not reuse</td>
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<tr>
<td>This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices</td>
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