Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, a reaction results between the native antigen and the antibody, forming an antibody-antigen complex. The interaction is illustrated by the following equation:

\[ \frac{k_c}{k_b} \text{ Rate Constant of Association} \]

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

\[ \text{Streptavidin } + \text{ Enzyme labeled antibody (Excess Quantity)} \]

\[ \text{Enzyme Antibody Complex} \]

\[ \text{Rate Constant of Dissociation} \]

4.0 REAGENTS

<table>
<thead>
<tr>
<th>Materials Provided:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. CA 15-3 Calibrators – 1 ml/vial - Ions ACF</td>
</tr>
<tr>
<td>Six (6) vials of patient based reference calibrators at concentrations of 0 (A), 10 (B), 40 (C), 100 (D), 200 (E) and 400 (F) U/ml. Store at 2-8°C. A preservative has been added.</td>
</tr>
<tr>
<td>Note 1: The calibrators are provided pre diluted.</td>
</tr>
<tr>
<td>Note 2: The calibrators, human serum based, were made using a purified preparation of CA 15-3 antigen. The preparation was calibrated against Centocor CA 15-3 IRMA test.</td>
</tr>
<tr>
<td>B. CA 15-3 Biotin Reagent – 12 ml/vial</td>
</tr>
<tr>
<td>One (1) vial contains biotinylated anti-human CA 15-3 mg/L in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.</td>
</tr>
<tr>
<td>C. CA 15-3 Enzyme Reagent – 12 ml/vial - Ions EC</td>
</tr>
<tr>
<td>One (1) vial contains horseradish peroxidase incorporate anti-human CA 15-3 mg/L in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.</td>
</tr>
</tbody>
</table>

D. Streptavidin Coated Plate – 96 wells -Ions EC |

E. Wash Solution Concentrate – 20 ml - Icon |

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1/2 and HIV antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum based calibrators should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institutes of Health, “Biosafety in Microbiological and Biomedical Laboratories,” 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum or heparinised plasma in type and taken with the usual precautions in the collection of venepuncture. An accurate comparison to establishment normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube or for plasma use evacuated tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the samples may be stored at -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the diluted specimen is required.

7.0 QUALITY CONTROL

Every laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should result in measured absorbance values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents.
10.0 CALCULATION OF RESULTS
A dose response curve is used to ascertain the concentration of CA15-3 in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding CA15-3 concentration in U/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of CA 15-3 for an unknown, locate the absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in U/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.721) intersects the dose response curve at (58.4 U/ml) CA 15-3 concentration (See Figure 1).

1.3.

12.0 RISK ANALYSIS
The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.
12.1 Assay Performance
1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of substrates should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolysed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose curve response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spuriously results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens (diluted) with CA 15-3 concentrations above 400 U/ml may be further diluted (1/10 or higher) with CA15-3 diluted serum diluent and re-assayed. The sample’s concentration is obtained by multiplying the result by the dilution factor.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind’s IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

12.2 Interpretation
1. Measurement and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. CA 15-3 has a low clinical specificity and sensitivity as a tumor marker. Clinically an elevated CA 15-3 value alone is not of diagnostic value as a test for cancer and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters.

13.0 EXPECTED RANGES OF VALUES
The serum CA 15-3 is elevated in 2% of normal healthy women and 7% of patients with non-neoplastic conditions. Also, it has been reported to be elevated in cases of liver, lung, ovarian and colorectal cancers. No definitive ranges have been reported for those conditions.

TABLE I

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration</th>
<th>Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA 15-3</td>
<td>1000 U/ml</td>
<td>1.000</td>
</tr>
<tr>
<td>CA 125</td>
<td>5000 U/ml</td>
<td>0.001</td>
</tr>
<tr>
<td>CA 19-9</td>
<td>1000 ng/ml</td>
<td>0.028</td>
</tr>
<tr>
<td>PSA</td>
<td>30,000 ng/ml</td>
<td>ND*</td>
</tr>
<tr>
<td>AFP</td>
<td>5,000 ng/ml</td>
<td>ND*</td>
</tr>
<tr>
<td>CEA</td>
<td>125,000 U/ml</td>
<td>ND*</td>
</tr>
<tr>
<td>HCG</td>
<td>12,500 IU/ml</td>
<td>0.001</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>200 mg/dl</td>
<td>ND*</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>30 ml/l</td>
<td>ND*</td>
</tr>
<tr>
<td>Lipid</td>
<td>50 mg/dl</td>
<td>-0.009</td>
</tr>
</tbody>
</table>

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analyst using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS
14.1 Precision
The within and between precision of the CA 15-3 assay is determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.2 Sensitivity
The CA 15-3 procedure has a analytical sensitivity of 0.2 U/ml at three (3) SD from the zero calibrator. The functional sensitivity (20% CV) was found to be 1.25 U/ml.

14.3 Accuracy
The Monobind CA 15-3 AccuBind™ ELISA procedure was compared with a reference Elisa method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 43. The least square regression equation and the correlation coefficient were computed for the CA 15-3 in comparison with the reference method. The data obtained is displayed in Table 4.

14.4 Specificity
In order to test the specificity of the antibody pair used massive combinations of possible cross-reactants were known serum pools and assayed in parallel with the base sera. No cross reaction was found. Percent cross-reacts for some of these additions are listed below in Table 5.

15.0 REFERENCES

Revision: 3  Data: 072611  Cat #: 5625-300  DCO:05054

For Orders and Inquiries, please contact

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On the Web: www.monobind.com

Please visit our website to learn more about our other interesting products and services.

Figure 1

Table 1

Sample | X | σ | C.V. |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>20.9</td>
<td>1.91 9.1%</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>61.7</td>
<td>2.03 3.3%</td>
</tr>
<tr>
<td>Level 3</td>
<td>20</td>
<td>104.6</td>
<td>9.33 8.9%</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate.