Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

\[ E_n + Ag_{CEA} + Ab_{CEA} \rightarrow E_n Ag_{CEA} Ab_{CEA} \]

\[ E_n Ag_{CEA} Ab_{CEA} \rightarrow E_n + Ag_{CEA} + Ab_{CEA} \]

\[ E_n = \text{Biotinylated Monoclonal Antibody (Excess Quantity)} \]

\[ Ag_{CEA} = \text{Native Antigen (Variable Quantity)} \]

\[ Ab_{CEA} = \text{Enzyme-labeled Antibody (Excess Quantity)} \]

\[ E_n Ag_{CEA} Ab_{CEA} = \text{Antigen-Antibodies Sandwich Complex} \]

\[ k_{as} = \text{Rate of Association} \]

\[ k_{ds} = \text{Rate of Dissociation} \]

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

\[ E_n + Ag_{CEA} + Ab_{CEA} \rightarrow E_n Ag_{CEA} Ab_{CEA} \]

\[ E_n Ag_{CEA} Ab_{CEA} \rightarrow E_n + Ag_{CEA} + Ab_{CEA} \]

\[ E_n + Ag_{CEA} + Ab_{CEA} \rightarrow E_n Ag_{CEA} Ab_{CEA} \]

\[ E_n = \text{Biotinylated Monoclonal Antibody (Excess Quantity)} \]

\[ Ag_{CEA} = \text{Native Antigen (Variable Quantity)} \]

\[ Ab_{CEA} = \text{Enzyme-labeled Antibody (Excess Quantity)} \]

\[ E_n Ag_{CEA} Ab_{CEA} = \text{Immobilized complex} \]

\[ Streptavidin_{cw} \rightarrow \text{Streptavidin immobilized on well} \]

\[ Immobilized complex = \text{sandwich complex bound to the well} \]

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

### 4.0 REAGENTS

**Materials Provided:**

A. **Next Generation Calibrators** – 1 ml vials

B. **Next Generation Enzyme Reagents**

C. **Streptavidin Coated Plate – 96 wells**

D. **Wash Concentrate**

E. **Substrate B**

F. **Stop Solution – 8ml/vial**

**5.0 QUALITY CONTROL**

Each laboratory should assure controls at levels in the low, normal and high range of values for an accurate determination of the amounts present in the sample. These controls should be treated as unknowns and values determined in the same manner as the test results. Determining the results of the unknowns as a percentage of normal values is not valid for this test. The results are read within thirty (30) minutes of adding the stop solution.

### 8.0 REAGENT PREPARATION

**1. Wash Buffer**

Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-4°C for up to 60 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

**2. Quality Control Materials**

**3. Stop Solution – 8ml/vial**

**4. Substrate B – 7ml/vial**

**5. Absorbent Paper for blotting the microplate wells.**

**6. Plastic wrap or microplate cover for incubation steps.**

**7. Microplate reader with 450nm and 620nm wavelength of light.**

**8. Computer data reduction software designed for ELISA assays**

**9. Quality control materials**

**10. Calculation of results**

A dose response curve is used to ascertain the concentration of CEA. The printed microplate reader is utilized. The printout of the microplate reader as outlined in Example 1.

**11. Quality control materials**

**Note:** Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

**Example 1**

<table>
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<th>Sample ID</th>
<th>Well Number</th>
<th>Abs (A)</th>
<th>Mean Abs (B)</th>
<th>Value (ng/ml)</th>
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</table>

*Note: The data presented in Example 1 and Table 1 are for illustration only and should not be used in lieu of a dose response curve prepared with each assay.*
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Boscato LM, Stuart MC. ‘Heterophilic antibodies: a problem for all immunoassays’. Clin Chem. 1988;34:27-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical criteria.

4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

5. 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 be liable.

6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the test results fall within 10% of the assigned concentrations.

7. CEA has a low clinical sensitivity and specificity as a tumor marker. Clinically, an elevated CEA value alone is not diagnostic of a test for cancer and should only be used in conjunction with other clinical manifestations (observations) of diagnostic parameters. There are patients with colorectal cancer that do not exhibit elevated CEA values and elevated CEA values do not always change with progression or regression of disease. Smokers demonstrate a higher range of baseline values than non-smokers.

11.0 EXPECTED RANGES OF VALUES

Nearly 99% of non-smokers have CEA concentrations less than 5 ng/ml. Similarly 99% of smokers have concentrations less than 10 ng/ml.

14.5 Linearity & Hook Effect:

Three different lot preparations of the CEA Next Generation AccuBind® ELISA reagents were used to assess the linearity and hook effect. Massive concentrations of CEA (≥60,000 ng/ml) were used for linear dilutions in pooled human patient sera.

The test showed no hook effect up to concentrations of 60,000 ng/ml and a within dose recovery of 92.0 to 111.4%.

15.0 REFERENCES