Carcinoembryonic Antigen (CEA)  
**Intended Use:** The Quantitative Determination of Carcinoembryonic Antigen (CEA) Concentration in Human Serum by a Microplate Immunoenzymometric assay  
  
**SUMMARY AND EXPLANATION OF THE TEST**  
Carcinoembryonic antigen (CEA) is a glycoprotein with a molecular weight of 180 kDa. CEA is the first of the so-called carcinoembryonic proteins that was discovered in 1965 by Gold and Freeman. CEA is the most widely used marker for gastrointestinal cancer.  

Although CEA is primarily associated with colorectal cancers, other malignancies that can cause elevated levels of CEA include breast, lung, stomach, pancreas, ovary and other organs. Benign conditions that cause significantly higher than normal levels include inflammation of lung and gastrointestinal (GI) tract and benign liver cancer (1,2). Heavy Smokers, as a group, have higher than normal baseline concentration of CEA.  

In this method, CEA calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against different and distinct epitopes of CEA) are added and the reagents mixed. Reaction between the various CEA antibodies and native CEA forms a sandwich complex that binds with the streptavidin coated to the well.  

After the completion of the required incubation period, the enzyme-CEA antibody bound conjugate is separated from the unbound enzyme-CEA conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.  

The employment of several serum references of known carcinoembryonic antigen (CEA) levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with CEA concentration.  

**PRINCIPLE**  
**Immunoenzymometric assay (TYPE 3):**  
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CEA antibody.  

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:  

\[
\text{EnzAb} + \text{Ag}_{\text{CEA}} + \text{BtnAb(m)} \xrightarrow{k_a} \text{EnzAb} - \text{Ag}_{\text{CEA}} - \text{BtnAb(m)}
\]

**PRECAUTIONS**  
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 HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products 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 Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.  

**SPECIMEN COLLECTION AND PREPARATION**  
The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain red top venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum 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 sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required.  

**QUALITY CONTROL**  
Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed changes in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.  

**REAGENT PREPARATION:**  
1. **Wash Buffer**  
   Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature (20-27°C) for up to 60 days.  

2. **Working Substrate Solution**  
   Pour the contents of the amber vial labeled Solution ‘A’ into the clear vial labeled Solution ‘B’. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2-8°C.  

   **Note:** Do not use the working substrate if it looks blue.  

**TEST PROCEDURE**  
Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).  

1. Format the microplates; wells for each serum reference, control and patient specimen to be assayed in duplicate.  
2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.  
3. Add 0.100 ml (100µl) of the CEA Enzyme Reagent to each well. It is very important to dispense all reagents close to the bottom of the coated well.  
4. Swirl the microplate gently for 20-30 seconds to mix and cover.  
5. Incubate 60 minutes at room temperature.  
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.  
7. Add 0.100 ml of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.  

   8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.  

   **DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION**  

9. Incubate at room temperature for fifteen (15) minutes.  

10. Add 0.050ml of stop solution to each well and mix gently for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.  

9. Incubate 60 minutes at room temperature.  

10. **Note:** The absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.  

   Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.  

   1. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.  

   2. Pipette 0.100 ml (100µl) of the CEA Enzyme Reagent to each well. It is very important to dispense all reagents close to the bottom of the coated well.  

   3. Add 0.100 ml (100µl) of the CEA Enzyme Reagent to each well.  

   4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.  

   5. Absorbent Paper for blotting the microplate wells.  

   6. Plastic wrap or microplate cover for incubation steps.  

   7. Vacuum aspirator (optional) for wash steps.  

   8. Timer.  

   9. Quality control materials  

   **Note:** Do not use reagents beyond the kit expiration date.  

   **Note:** Always add reagents in the same order to minimize reaction time differences between wells.  

   **DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION**  

10. Incubate at room temperature for fifteen (15) minutes.  

11. Add 0.050ml of stop solution to each well and mix gently for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.  

   **Note:** Do not use reagents beyond the kit expiration date.  

   **Note:** Always add reagents in the same order to minimize reaction time differences between wells.  

11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.
A dose response curve is used to ascertain the concentration of Carcinoembryonic antigen 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 following criteria should be met:  
   1. It is important that the time of reaction in each well is held constant for reproducible results.  
   2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.  
   3. If more than one (1) plate is used, it is recommended to repeat the dose response curve.  
3. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution.  
Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time-deviation during reaction.  
4. Plate readers measure vertically. Do not touch the bottom of the wells.  
5. Failure to remove adhering solution adequately in the aspiration wash step(s) may result in poor replication and spurious results.  
6. Use components from the same lot. No intermixing of reagents from different batches.  
7. Use components with normal male serum (CEA < 5 ng/ml) and re-assayed. The sample’s concentration is obtained by multiplying the result by the dilution factor (10).  

**EXPECTED RANGES OF VALUES**  
Nearly 99% of non-smokers have CEA concentrations less than 5ng/ml. Similarly 99% of smokers have concentrations less than 10ng/ml.

**REFERENCES**  

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