Tribo™ Human CEA ELISA Kit (Catalog# TBS3210)

DESCRIPTION

The Tribo™ Human Carcinoembryonic Antigen ELISA Kit is designed for in vitro quantitative determination of CarcinoEmbryonic Antigen (CEA) concentrations in human serum and plasma samples. This assay employs an antibody specific for Human Carcino Embryonic Antigen CEA coated on a 96-well plate. Standards and samples are pipetted into the wells and Carcino Embryonic Antigen CEA present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-Human Carcino Embryonic Antigen CEA antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted into the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Carcino Embryonic Antigen CEA bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450nm. The concentration of CEA in the samples is calculated opposite to the standard curve.

APPLICATIONS

Direct Assays: quantitative CEA in different tissues.

MAIN FEATURES

Sensitivity: The minimum detectable dose of human CEA is typically less than 2.0 pg/mL.
Specificity: This assay recognizes recombinant and natural human CEA. No cross-reactivity with any other cytokine is observed.

KIT CONTENTS

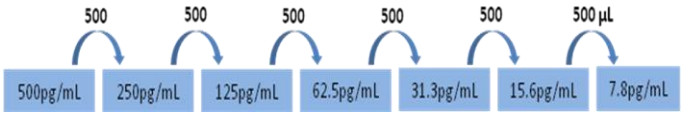
Reagents	Quantity
Precoated 96-well Plate (8x 12 strips)	1 plate
Standard	2x 100µL
Detection A	1x 100µL
Detection B	1x 100µL
Sample Diluent (10x concentrate)	1x 12ml
TMB Substrate Solution	1x 12ml
Stop Solution	1x 12ml
Wash Solution (20x concentrate)	1x 50ml
Plate Sealers	4

Storage conditions: Store the kit at 4°C and standard at -20°C, respectively. Shelf life: 12 months after receipt.

PROCEDURES

Reagent Preparation:

1. Bring all reagents to room temperature (18-25˚C) before use. Briefly spin down all small tubes.
2. Wash Buffer: Add 50mL of 20x concentrated Wash Buffer into 950mL distilled water and mix thoroughly.
3. Reagent Diluent: Dilute 10mL of 10x concentrated Sample Diluent with 90mL of deionized water (1:10) before use.
4. Dilute Detection A: Add 100µL of the Detection A into 10mL of the Reagent Diluents for 96-well plate.
5. Dilute Detection B: Add 100µL of the Detection B into 10mL of the Reagent Diluents for 96-well plate.
6. Standard: Set up 7 points of CEA standard concentrations: 500, 250, 125, 62.5, 31.3, 15.6 and 7.8 pg/mL as shown in the diagram below.
7. Label 7 eppendoule tubes from 1-7. Add 900µL of the Reagent Diluents to the tube #1. Add 500µL of the Reagent Diluents to tubes #2-7.
8. Add 100 µL of the CEA standard into tube #1 and vortex. This is Standard tube #1 with a concentration of 500 pg/mL.
9. Standards #2-7 are then prepared by performing a 1:2 dilution of the preceding standard. Mix each tube thoroughly before the next transfer. For example, to make Standard #2, remove 500µL of Standard #1 and add it to tube #2 and vortex and so on. The Reagent Diluents serves as the zero standard (0 pg/ml).



Test Procedures:

1. Add 100µL of standards, blank and sample into plate in duplicate manner. Seal the plate and incubate for 2 hrs at room temperature.
2. Remove sealer and empty wells. Wash the plate 3 times with 300µL of Wash Buffer.
3. Add 100µL of the diluted Detection A to each well. Seal the plate and incubate for 2 hrs at room temperature.

4. Repeat washing step 2.
5. Add 100µL of the diluted Detection B to each well. Seal the plate and incubate for 20 min at RT. Protect from light.
6. Repeat washing Step 2.
7. Add 100µL of TMB Substrate Solution to each well. Incubate for 10-20 min at RT. Protect from light.
8. Add 50µL of Stop Solution to each well when the highest standard has developed a dark blue color. Gently tap the plate to ensure thoroughly mixing.
9. Read the optical density of each well immediately, using a microplate reader set to 450nm.

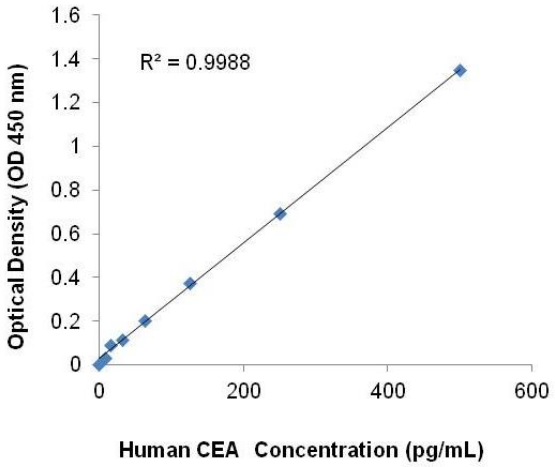
CALCULATION

Average the duplicate readings for each standard, blank and samples, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best-fit curve through the points on the graph. The data may be linearized by plotting the log of the human CEA concentrations versus the log of the O.D. and the best-fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

- Microplate reader
- Pipettes and Pipette tips
- Microtubes

Standard Curve of Human CEA



REFERENCES

1. Khoo SK and Mackay FR. Carcinoembryonic antigen in serum in diseases of the liver and pancreas. J. Clin. Path. 1973; 26: 470-475
2. Laurence DJR, Stevens U, and Bellelheim R, et al. Evaluation of the role of carcinoembryonic antigen in the diagnosis of gastro-intestinal, mammary and bronchial carcinoma. Br. Med. J. 1972, 3: 605-609.

RELATED PRODUCTS:

Tribo™ Human AFP ELISA (Catalog# TBS3212)

Tribo™ Human HE4 ELISA (Catalog# TBS3213)

Tribo™ Human CA125 ELISA (Catalog# TBS3214)

Tribo™ Human CA19-9 ELISA (Catalog# TBS3215)

Tribo™ Human CA15-3 ELISA (Catalog# TBS3216)

Tribo™ Human PSA ELISA (Catalog# TBS3217)

Tribo™ Human ß2-microglobulin ELISA (Catalog# TBS3218)

Tribo™ Fast Human IFN- γ ELISA (Catalog# TBS3230)

Protein Cell Lysis Buffer (Catalog# TBS5001)

Protein Assay Kit (Catalog# TBS2005)

TMB Substrate System (Catalog# TBS5021)

365 San Aleso Ave, Sunnyvale, CA 94085, USA Website: www.tribioscience.com
Tel: 408-423-9096, Fax: 650-618-5498 Email: order@tribioscience.com