Human Carcinoembryonic Antigen (CEA) ELISA Kit

Cat. No.: EJ1514330 | Pack size: 48T / 96T | Storage: 2-8 °C

Overview

Carcinoembryonic Antigen (CEA) refers to a group of highly homologous glycoproteins involved in cell adhesion. It is normally synthesized in gastrointestinal tissues during fetal development and ceases production before birth, resulting in low serum CEA concentrations (approximately 2-4 ng/mL) in healthy adults. Nevertheless, serum CEA levels rise in certain malignancies, qualifying CEA as a tumor biomarker for clinical testing. Elevated CEA can also be detected in heavy smokers. As a GPI-anchored cell-surface glycoprotein, CEA bears specific fucosylated glycoforms that serve as functional ligands for L-selectin and E-selectin in colon carcinoma, playing a critical role in colon cancer cell migration and metastasis. Immunologically, CEA belongs to the CD66 cluster of differentiation family, comprising CD66a, CD66b, CD66c, CD66d, CD66e and CD66f.

Assay Principle

This kit adopts sandwich enzyme-linked immunosorbent assay (ELISA). Microwells pre-coated with anti-human CEA capture antibody are sequentially loaded with samples, standards, Biotin-antibody and Streptavidin-HRP following incubation and washing steps. Color development is triggered by TMB substrate: TMB turns blue under HRP catalysis and shifts to yellow upon acid termination. Color intensity is positively correlated with human CEA concentration in specimens. Absorbance (OD value) is measured at 450 nm via microplate reader to calculate sample analyte concentration.

Product Information

Item	Information
Grade & Purity	BioReagent; for Enzyme immunoassay (ELISA)
Storage	Store at 2-8 °C.
Shipped In	Wet ice
Stability and Storage	Store at 2-8 °C long term (6 months).
Available Quality Documents	SDS, COA, datasheet, and spec sheet are available. Lot-specific COA is accessible via lot number lookup.

Contents & Storage

Cat. No.	Components	Appearance	48T	96T	Storage
EJ1514330A	Pre-coated Assay Plate	—	48 wells	96 wells	2-8 °C.
EJ1514330B	Standard	Solid	1 vial	2 vials	2-8 °C.
EJ1514330C	Universal Diluent	Liquid	20 mL	2×20 mL	2-8 °C.
EJ1514330D	Biotin-antibody (100×)	Liquid	60 µL	120 µL	2-8 °C.
EJ1514330E	Streptavidin-HRP (100×)	Liquid	60 µL	120 µL	2-8 °C.
EJ1514330F	Wash Buffer (20×)	Liquid	10 mL	2×10 mL	2-8 °C.
EJ1514330G	TMB Substrate	Liquid	5 mL	10 mL	2-8 °C.
EJ1514330H	Stop Solution	Liquid	3 mL	6 mL	2-8 °C.
EJ1514330I	Plate Sealer	—	4 pieces	4 pieces	2-8 °C.

Note: EJ1514330B, EJ1514330D, EJ1514330E and EJ1514330F shall be diluted in accordance with the instructions.

Precautions

- Incubate strictly at specified time and temperature to ensure reliable test results. Equilibrate all reagents to ambient temperature (20-25 °C) prior to use; refrigerate reagents promptly after usage.
- Improper plate washing causes erroneous readings. Remove residual liquid completely from wells before substrate addition; avoid prolonged desiccation of microwells throughout the assay.
- Wipe off residual liquid and fingerprints on the bottom of microplate to prevent OD interference.
- Fresh TMB substrate solution shall be colorless; discard blue-discolored substrate.
- Prevent cross-contamination between reagents and samples to avoid invalid experimental data.
- Shield reagents from direct intense light during storage and incubation.
- All assay reagents must avoid contact with bleach solution or volatile fumes from bleach, as bleach ingredients will inactivate biological activity of kit contents.
- Expired products are prohibited for use; components from different catalog numbers or batch lots cannot be mixed.
- Exogenous recombinant proteins from non-kit sources may fail antibody recognition due to epitope mismatch.
- All biohazardous specimens and testing consumables shall be managed and disposed of in accordance with institutional biosafety protocols.

Sample Pretreatment and Requirements

- The detection range of the kit is not equivalent to the analyte concentration range in samples. It is recommended to estimate the analyte concentration via relevant literature before experiment and confirm the actual concentration by pre-experiment. Properly dilute or concentrate samples with excessively high or low analyte concentrations.
- If the sample type is not listed in the manual, perform a pre-experiment to verify detection feasibility.
- Serum: Let whole blood collected in serum separator tubes stand at room temperature for 2 hours or at 2-8 °C overnight, then centrifuge at 1000×g for 20 min to collect supernatant. Store supernatant at -20 °C or -80 °C and avoid repeated freeze-thaw cycles.
- Plasma: Collect blood with EDTA or heparin as anticoagulant. Centrifuge specimens at 1000×g for 15 min within 30 min after collection under 2-8 °C, collect supernatant for immediate testing or store at -20 °C/-80 °C without repeated freeze-thaw.
- Tissue homogenate: Rinse fresh tissue with pre-cooled PBS (0.01 M, pH=7.4) to remove residual blood as lysed red blood cells interfere detection. Weigh and mince tissue, add minced tissue into glass homogenizer with PBS at 1:9 weight-to-volume ratio (1 g tissue with 9 mL PBS; adjust volume as needed and record; protease inhibitor is recommended in PBS). Grind fully on ice or with homogenizer. Further lyse cells by sonication or repeated freeze-thaw, then centrifuge homogenate at 5000×g for 5-10 min and collect supernatant.
- Cell culture supernatant: Centrifuge at 1000×g for 20 min, collect supernatant for test or store at -20 °C/-80 °C avoiding repeated freeze-thaw.
- Cell lysate: Wash adherent cells with pre-cooled PBS, digest with trypsin and harvest by 1000×g centrifugation for 5 min; collect suspension cells directly by centrifugation. Wash harvested cells 3 times with pre-cooled PBS, resuspend 1×10^6 cells in 150-200 µL PBS (protease inhibitor recommended; reduce PBS volume for low-abundance targets). Break cells via repeated freeze-thaw or sonication, centrifuge lysate at 1500×g for 10 min at 2-8 °C and collect supernatant.
- Other biological samples: Centrifuge at 1000×g for 20 min and retain supernatant.
- Sample appearance: Samples shall be clear; remove suspended sediments by centrifugation.
- Sample storage: Specimens can be kept at 4 °C within 7 days after collection. Aliquot unused samples and freeze at -20 °C (valid within 1 month) or -80 °C (valid within 6 months) to avoid repeated freeze-thaw. Hemolyzed samples are unsuitable for detection.

Sample Dilution Protocol

Estimate sample concentration in advance and dilute as required referring to below methods: 100-fold dilution: Single-step dilution, mix 5 µL sample with 495 µL Universal Diluent. 1000-fold dilution: Two-step dilution, first mix 5 µL sample with 95 µL Universal Diluent for 20× dilution, then take 5 µL diluted solution into 245 µL Universal Diluent for 50× dilution to reach total 1000-fold. 100000-fold dilution: Three-step dilution, first mix 5 µL sample with 195 µL Universal Diluent for 40× dilution;

second take 5 µL diluted liquid into 245 µL diluent for 50× dilution; third take 5 µL twice-diluted sample into 245 µL diluent for another 50× dilution to reach total 100000-fold. Minimum pipetting volume per dilution ≥3 µL and single dilution fold ≤100. Mix thoroughly without bubbles after each dilution.

Required Laboratory Supplies

- Microplate reader (450 nm).
- Precision pipettes and tips: 0.5-10 µL, 5-50 µL, 20-200 µL, 200-1000 µL.
- 37 °C incubator.
- Distilled or deionized water.

Pre-Assay Reagent Preparation

1. Take kit out from refrigerator 10 min earlier and equilibrate to room temperature.

2. Preparation of serial standard working solution: Reconstitute lyophilized standard with 1 mL Universal Diluent, leave for 15 min to fully dissolve and mix gently (stock concentration: 1250 pg/mL). Prepare serial concentrations: 1250 pg/mL, 625 pg/mL, 312.5 pg/mL, 156.2 pg/mL, 78.1 pg/mL, 39.06 pg/mL, 19.53 pg/mL, 0 pg/mL. Serial dilution operation: Prepare 7 EP tubes pre-filled with 500 µL Universal Diluent each. Transfer 500 µL 1250 pg/mL standard into the first tube to get 625 pg/mL solution, perform successive half-dilution sequentially. The last tube serves as blank control without adding standard liquid.

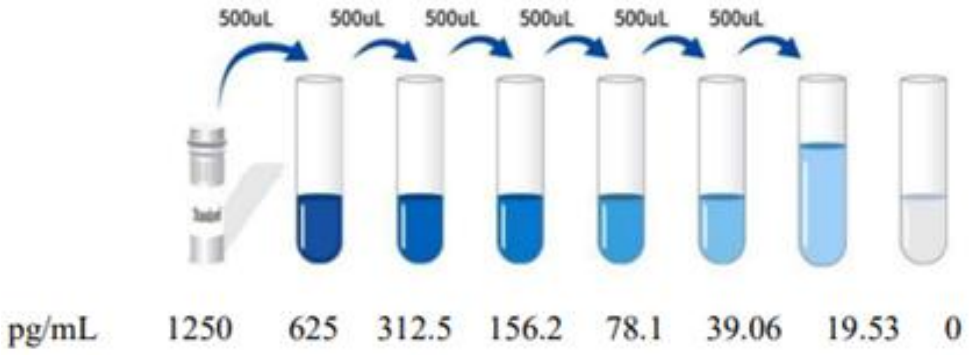

Figure 1. Standard dilution series.

3. Preparation of Biotin-antibody working solution: Centrifuge 100× concentrated Biotin-antibody at 1000×g for 1 min 15 min before use. Dilute the concentrated stock to 1× working concentration with Universal Diluent (Example: 10 µL concentrate + 990 µL Universal Diluent). Prepare freshly before use.

4. Preparation of Streptavidin-HRP working solution: Centrifuge 100× concentrated Streptavidin-HRP at 1000×g for 1 min 15 min before use. Dilute the concentrated stock to 1× working concentration

with Universal Diluent (Example: 10 µL concentrate + 990 µL Universal Diluent). Prepare freshly before use.

5. Preparation of 1× Wash Buffer: Add 10 mL of 20× concentrated Wash Buffer into 190 mL distilled water. Crystals may form in concentrated Wash Buffer stored under refrigeration, which is normal. Let the buffer stand at room temperature until crystals dissolve completely prior to dilution.

Assay Procedure

Take required strips from the aluminum pouch equilibrated at room temperature for 10 min. Seal unused strips in zip lock bag and store at 4 °C.

Sample loading: Pipette 100 µL of samples or serially diluted standards into respective wells; add 100 µL Universal Diluent into blank well. Seal plate and incubate at 37 °C for 60 min. Recommendation: Dilute test samples no less than 1-fold with Universal Diluent to minimize matrix effect; multiply final concentration by corresponding dilution factor during calculation. Running duplicate wells is recommended for all samples and standards.

Add Biotin-antibody: Discard well contents without washing. Add 100 µL prepared Biotin-antibody working solution per well, seal plate and incubate at 37 °C for 60 min.

Plate washing: Decant liquid, fill each well with 300 µL 1× Wash Buffer, soak for 1 min, tap dry on absorbent paper. Repeat wash cycle for total 3 times; automatic plate washer is optional.

Add Streptavidin-HRP working solution: Dispense 100 µL Streptavidin-HRP working solution per well, seal plate and incubate at 37 °C for 30 min.

Plate washing: Remove liquid and repeat washing procedure described in step 4 for total 5 cycles.

Substrate addition: Add 90 µL TMB substrate into each well, seal plate and incubate at 37 °C in dark for 15 min.

Stop reaction: Add 50 µL Stop Solution to every well and immediately measure OD value at 450 nm.

Calculation of Test Results and Result Interpretation

Calculate average OD of duplicate wells for standards and samples, subtract blank OD to obtain corrected OD values. Plot 4-parameter logistic standard curve on log-log graph with concentration on X-axis and corrected OD on Y-axis.

Retest samples with OD above upper limit of standard curve after appropriate dilution and correct final concentration with relevant dilution factor.

Typical Data & Reference Standard Curve

The listed data and curve are for reference only; each laboratory shall establish its own standard curve per assay run.

Concentration (pg/mL)	1250	625	312.5	156.2	78.1	39.06	19.53	0
OD Value	2.05	1.12	0.57	0.35	0.25	0.20	0.18	0.08
Corrected OD Value	1.97	1.04	0.49	0.27	0.17	0.12	0.10	-

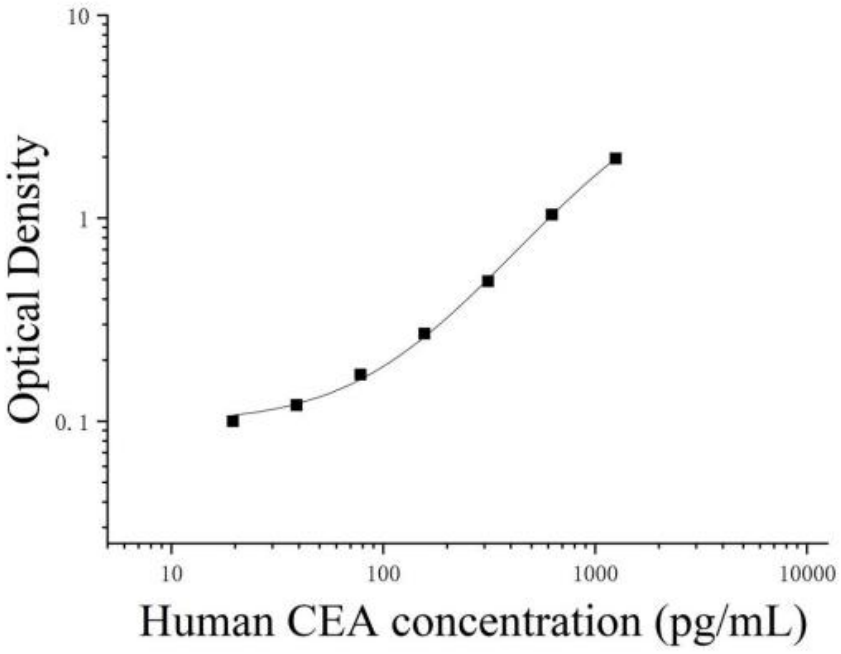

Figure 2. Reference standard curve for human CEA.

Note: This figure is for reference only; sample concentration shall be calculated based on the standard curve generated from experimental data of each individual test run.

Kit Performance

1. Precision: Intra-assay CV <10%, Inter-assay CV <10%.

2. Recovery: Three concentration levels of human CEA were spiked into healthy human serum, plasma and cell culture supernatant to calculate recovery rate.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=8)	84-101	96
Plasma (n=8)	92-105	102
Cell Culture Supernatant (n=8)	96-108	105

3. Linearity on dilution: High-concentration human CEA was spiked into 4 batches of healthy human serum, plasma and cell culture supernatant respectively, followed by serial dilution within the dynamic range of standard curve to evaluate linearity.

Dilution Ratio	Recovery (%)	Serum	Plasma	Cell Culture Supernatant
1:2	Range	84-95	88-96	90-110
1:2	Average Recovery	91	93	95
1:4	Range	89-103	87-108	105-115
1:4	Average Recovery	94	98	108

Quality Control

QC Item	Method	Acceptable Range
Standard curve fit	4-parameter logistic regression or equivalent model using the standard data of each run	Curve fit suitable for interpolation across the validated standard range
Precision	Coefficient of variation across replicate wells or runs	Intra-assay CV <10%; Inter-assay CV <10%
Component completeness	Visual inspection on receipt	All components and counts match the kit contents table; labels and lot number are legible

Troubleshooting Guide

If unsatisfactory assay results occur, take photos of color development, save experimental data, retain used microplate strips and unused reagents, then contact our technical support for troubleshooting. Refer to the troubleshooting items below:

1. Poor standard curve linearity

① Incorrect standard dilution: Reconstitute and dilute standard strictly following recommended protocol.

② Inaccurate pipetting: Calibrate pipettes regularly and verify tip tightness/sealing performance.

③ Evaporation of reaction solution: Seal microplate with plate sealer film.

④ Incomplete washing: Perform sufficient wash cycles with adequate volume of wash buffer.

⑤ Foreign residues at well bottom: Wipe microplate bottom prior to absorbance measurement.

2. Faint or absent color development

① Insufficient incubation duration: Adhere to specified incubation time.

② Incorrect incubation temperature: Incubate at recommended temperature.

③ Insufficient reagent addition volume: Verify pipette accuracy and follow operating procedure strictly.

④ Improper reagent dilution: Double-check dilution steps for all reagents.

⑤ Inactivated Streptavidin-HRP: Mix Streptavidin-HRP with substrate and verify activity via color reaction test.

3. Low OD readings

① Wrong microplate reader parameter: Confirm detection wavelength setting on instrument.

② Missing stop solution addition: Add appropriate volume of stop solution.

③ Overlong delay before plate reading: Measure absorbance immediately after termination.

④ Excessively high analyte concentration in sample: Determine optimal dilution factor via preliminary pre-test.

⑤ Too low analyte concentration in sample: Determine optimal dilution factor via preliminary pre-test.

6. High background signal

① Contaminated TMB substrate: Replace with fresh substrate solution.

② Excess substrate incubation time: Strictly control color development duration.

③ Wrong dilution for detection antibody or Streptavidin-HRP: Dilute reagents in accordance with official recommended protocol.

④ Incomplete plate washing: Carry out adequate wash cycles with sufficient wash buffer volume.

Recommended Applications

Quantitative measurement of human CEA in research samples, including serum, plasma, tissue homogenate, cell culture supernatant, cell lysate, and other biological samples. This product is intended for research use only.

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Performance depends on sample type, sample condition, handling, and operator technique. Users are responsible for validating the kit for their specific application.

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