N-epsilon-Carboxyethyl Lysine ELISA Kit

Enzyme Immunoassay for the quantification of N-epsilon-Carboxyethyl Lysine in purified protein, plasma, serum, or cell lysate.

Catalog number: ARG81229

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

N ε-Carboxyethyl lysine (CEL), also known as N-epsilon-carboxyethyl lysine, is an advanced glycation endproduct (AGE). CEL has been the most used marker for AGEs in food analysis. [Provide by Wikipedia: CEL]

Advanced glycation end products (AGEs) are proteins or lipids that become glycated as a result of exposure to sugars. They are a bio-marker implicated in aging and the development, or worsening, of many degenerative diseases, such as diabetes, atherosclerosis, chronic kidney disease, and Alzheimer's disease. AGEs affect nearly every type of cell and molecule in the body and are thought to be one factor in aging and some age-related chronic diseases. They are also believed to play a causative role in the vascular complications of diabetes mellitus. AGEs arise under certain pathologic conditions, such as oxidative stress due to hyperglycemia in patients with diabetes. AGEs play a role as pro-inflammatory mediators in gestational diabetes as well. [Provide by Wikipedia: Advanced glycation end-product]

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. First, a CEL conjugate is coated on the protein binding plate. The CEL protein samples or standards (CEL-BSA standards) are then added to the CEL conjugate pre-coated plate. After a brief incubation, the Antibody Conjugate is added. The CEL conjugate competes with the CEL protein of the samples / standards for the limited number of antibody sites. After incubation, the wells are washed with wash buffer to remove unbound material. After adding an HRP Conjugate for incubation, the wells are washed with wash buffer to remove unbound material. Then the TMB substrate is added to the wells and color develops in proportion to the amount of CEL protein adduct competition in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm. The concentration of CEL protein adduct in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, aliquot and store the Antibody Conjugate, Standards, CEL conjugate, and 100X Conjugate Diluent at -20°C to avoid multiple freeze/thaw cycles. Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Protein Binding microplate	8 X 12 strips	4°C
Standards (1 mg/ml CEL-BSA in PBS)	30 µL	–20°C
CEL Conjugate (1 mg/ml in PBS)	20 µL	–20°C
100X Conjugate Diluent	300 µL	–20°C
Diluent Buffer	50 ml (ready to use)	4°C
Antibody Conjugate (Anti-CEL Antibody, 1000X)	10 µL	–20°C
HRP Conjugate Antibody (1000X)	20 µL	4°C
10X Wash Buffer	100 ml	4°C
TMB substrate	12 ml (ready to use)	4°C (protect from light)
STOP solution	12 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- 1X PBS
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- The Stock Standards, Antibody Conjugate, CEL Conjugate and Conjugate Diluent should be aliquoted into smaller portions before use to ensure product integrity and store the aliquoted material above at -20°C. Avoid repeated freeze-thaw cycles
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB

solution to glass, foil or metal. If the solution is blue before use, do NOT use it.

- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Use a new adhesive plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Collect the plasma layer and store on ice.

Cells and tissue lysate: Homogenize 50-200 mg of the cell and tissue pellet in 0.5-2 mL of ice cold PBS using a mortar and pestle or by dounce homogenization. Incubate the homogenate at 4°C for 20 minutes. Transfer the homogenate to a centrifuge tube and centrifuge at 12000 x g for 20 minutes. Recover the supernatant and transfer to a fresh tube. Store resuspended sample at -20°C or colder until ready to test by ELISA.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum/plasma sample.
3. Aliquot samples for testing and store at -80°C. Avoid repeated freeze-thaw cycles. Perform dilutions in Diluent buffer as necessary.
4. Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- CEL Conjugate Coated Plate:
    1. Immediately before use, prepare 1X Conjugate Diluent by diluting the 100X Conjugate Diluent in 1X PBS. Example: Add 50 µL to 4.95 mL of 1X PBS.
    2. Immediately before use, prepare 1.0 µg/mL of CEL Conjugate by diluting the 1.0 mg/mL CEL Conjugate in 1X Conjugate Diluent. Example: Add 5 µL of 1.0 mg/mL CEL Conjugate to 4.995 mL of 1X Conjugate Diluent and mix well.
    3. Add 100 µL of the 1 µg/mL CEL Conjugate to each well to be tested and incubate overnight at 4°C. Remove the CEL Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Diluent Buffer to each well and block for 1 hour at RT on a microplate shaker. Transfer the plate to 4°C and remove the Diluent Buffer immediately before use.

    Note: The CEL Conjugate coated wells are not stable and should be used within 24 hours after coating. Only coat the number of wells to be used immediately.
- 1X Wash Buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (E.g., add 50 ml of 10X Wash buffer into 450 ml of distilled water to a final volume of 500 ml) The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C.
- Antibody Conjugate and HRP Conjugate antibody: Immediately before use dilute the Antibody Conjugate (Anti-CEL antibody) 1:1000 and the HRP Conjugate Antibody 1:1000 with Diluent Buffer. Do not store diluted

solutions.

- **Standards (CEL-BSA standards):** Prepare a series dilution of CEL-BSA standards. The Diluent Buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted with Diluent Buffer as according to the suggested concentration table below:

Standard tubes	Final CEL-BSA conc. (ng/mL)	Diluent Buffer (µL)	Standards (µL)
S1	25	390	10 of 1 mg/ml CEL-BSA standards
S2	12.5	200	200 of S1
S3	6.25	200	200 of S2
S4	3.13	200	200 of S3
S5	1.56	200	200 of S4
S6	0.78	200	200 of S5
S7	0.39	200	200 of S6
S8	0.20	200	200 of S7
S9	0.10	200	200 of S8
S0	0	200	0

Note: Dilutions for the standard must be made and applied to the plate immediately. S0 serves as background.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. If testing mouse or rat plasma or serum, the IgG must be completely removed from each sample prior to testing, such as with Protein A or G beads. Additionally, a control well without Antibody Conjugate should be run for each sample to determine background signal.

1. Add **50 µL** of **sample or Standards** to the **CEL Conjugate Coated microplate.** If needed, **samples** may be diluted in **1X PBS containing 0.1% BSA** before adding.
2. Incubate at **RT** for **10 mins** on a microplate shaker.
3. Add **50 µL** of the **diluted Antibody Conjugate** to each well, incubate at **RT** for **1 hour** on a microplate shaker.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µL** of the **diluted HRP Conjugate** to each well. Incubate at **RT** for **1 hour** on a microplate shaker.
6. Aspirate each well and **wash as step 4**.
7. Warm **TMB Substrate** to **RT**. Add **100 µl** of **TMB Substrate** to each well, including the blank wells. Incubate for **5-20 minutes** on a microplate shaker at **RT** in the dark.

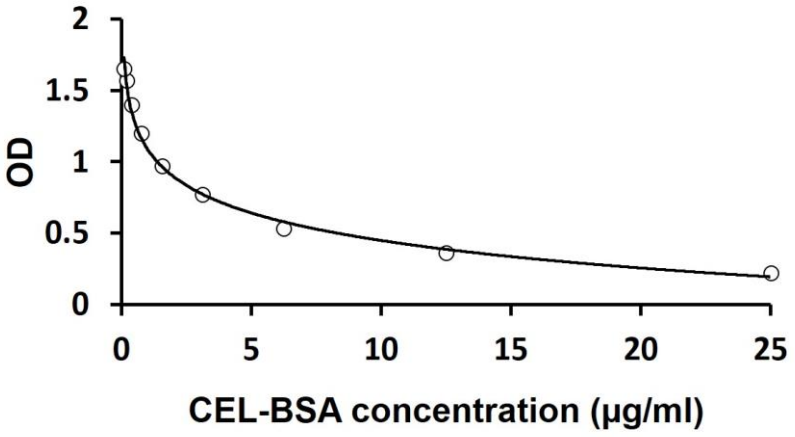
   Note: Watch plate carefully; if color changes rapidly, the reaction may need

to be stopped sooner to prevent saturation.

8. Add 100 µl of Stop Solution to each well, including the blank wells. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450nm immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance within 30 minutes after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the N-epsilon-Carboxyethyl Lysine ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Intra-assay and Inter-assay precision

The CV value of intra-assay and inter-assay precision was ≤ 10%.

Sensitivity

0.1 µg/ml