Product Manual

OxiSelect™ Nε-(carboxyethyl) lysine (CEL) Competitive ELISA Kit

Catalog Number

STA-813	96 assays
STA-813-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

The non-enzymatic reaction of reducing carbohydrates with lysine side chains and N-terminal amino groups of macromolecules (proteins, phospholipids and nucleic acids) is called the Maillard reaction or glycation. The products of this process, termed advanced glycation end products (AGEs), adversely affect the functional properties of proteins, lipids and DNA. Tissue levels of AGE increase with age and the formation of AGEs is predominantly endogenous, though these products can also be derived from exogenous sources such as food and tobacco smoke. AGE modification of proteins can contribute to the pathophysiology of aging and long-term complications of diabetes, atherosclerosis and renal failure. AGEs also interact with a variety of cell-surface AGE-binding receptors (RAGE), leading either to their endocytosis and degradation or to cellular activation and pro-oxidant or pro-inflammatory events.

Although several AGE structures have been reported, it was demonstrated that N^{ε}-(carboxymethyl) lysine (CML) and N^{ε}-(carboxyethyl) lysine (CEL) are the major antigenic AGE structures. Next to glucose, reactive di-carbonyl compounds such as methylglyoxal are major precursors in the formation of cellular and extracellular AGEs. Methylglyoxal reacts with lysine residues to form CEL. CEL concentration is increased in patients who have diabetes with complications.

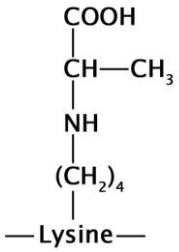

Figure 1. Structure of N^{ε}-(carboxyethyl) lysine (CEL)

OxiSelect™ N^{ε}-(carboxyethyl) lysine (CEL) ELISA Kit provides rapid detection and quantitation of CEL protein adducts. The quantity of CEL adduct in protein samples is determined by comparing its absorbance with that of a known CEL-BSA standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown protein samples.

Assay Principle

First, a CEL conjugate is coated on the ELISA plate. The unknown CEL protein samples or CEL-BSA standards are then added to the CEL conjugate preabsorbed plate. After a brief incubation, the anti-CEL monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The content of CEL protein adducts in unknown samples is determined by comparison with the predetermined CEL-BSA standard curve. Despite the structure similarity between CEL and CML, the anti-CEL specific antibody in the OxiSelect™ CEL ELISA Kit will not cross react with CML protein adducts.

Related Products

1. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
2. STA-307: OxiSelect™ Protein Carbonyl Flurometric Assay
3. STA-308: OxiSelect™ Protein Carbonyl Immunoblot Kit
4. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
5. STA-811: OxiSelect™ Methylglyoxal (MG) Competitive ELISA Kit
6. STA-816: OxiSelect™ N^{ε}-(carboxymethyl) lysine (CML) Competitive ELISA Kit
7. STA-817: OxiSelect™ Advanced Glycation End Products (AGE) Competitive ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. Anti-CEL Antibody (1000X) (Part No. 281301): One 10 µL vial of anti-CEL antibody.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. CEL-BSA Standard (Part No. 281302): One 30 µL vial of 1 mg/mL CEL-BSA in PBS.
2. CEL Conjugate (Part No. 281303): One 20 µL vial of CEL conjugate at 1.0 mg/mL in PBS.
3. 100X Conjugate Diluent (Part No. 281603): One 300 µL vial.

Materials Not Supplied

1. Protein samples such as purified protein, plasma, serum, cell lysate
2. 1X PBS
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Anti-CEL Antibody, CEL-BSA Standard, CEL Conjugate and 100X Conjugate Diluent at -20ºC to avoid multiple freeze/thaw cycles. Store all other kit components at 4ºC.

Preparation of Reagents

- CEL Conjugate Coated Plate:

  Note: The CEL Conjugate coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.

  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 4ºC. 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 Assay Diluent to each well and block for 1 hr at room temperature on an orbital shaker. Transfer the plate to 4ºC and remove the Assay Diluent **immediately before use.**

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-CEL Antibody and Secondary Antibody: Immediately before use, dilute the Anti-CEL antibody 1:1000 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of CEL-BSA standards in the concentration range of 0 to 25 µg/mL by diluting the 1.0 mg/mL CEL-BSA standard in Assay Diluent (Table 1).

Standard Tubes	1 mg/mL CEL-BSA Standard (µL)	Assay Diluent (µL)	CEL-BSA (µg/mL)
1	10	390	25
2	200 of Tube #1	200	12.5
3	200 of Tube #2	200	6.25
4	200 of Tube #3	200	3.13
5	200 of Tube #4	200	1.56
6	200 of Tube #5	200	0.78
7	200 of Tube #6	200	0.39
8	200 of Tube #7	200	0.20
9	200 of Tube #8	200	0.10
10	0	200	0

Table 1. Preparation of CEL-BSA Standard Curve

Assay Protocol

Note: 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 primary antibody should be run for each sample to determine background signal.

1. Prepare and mix all reagents thoroughly before use. Each CEL sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of the unknown sample or CEL-BSA standard to the wells of the CEL Conjugate coated plate. If needed, unknown samples may be diluted in 1X PBS containing 0.1% BSA before adding. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 µL of the diluted anti-CEL antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash 3 times with 250 µL of 1X Wash Buffer with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Secondary Antibody-HRP Conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 3 times according to step 4 above.
6. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well. Incubate at room temperature for 2-30 minutes on an orbital shaker.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
7. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).
8. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical CEL ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

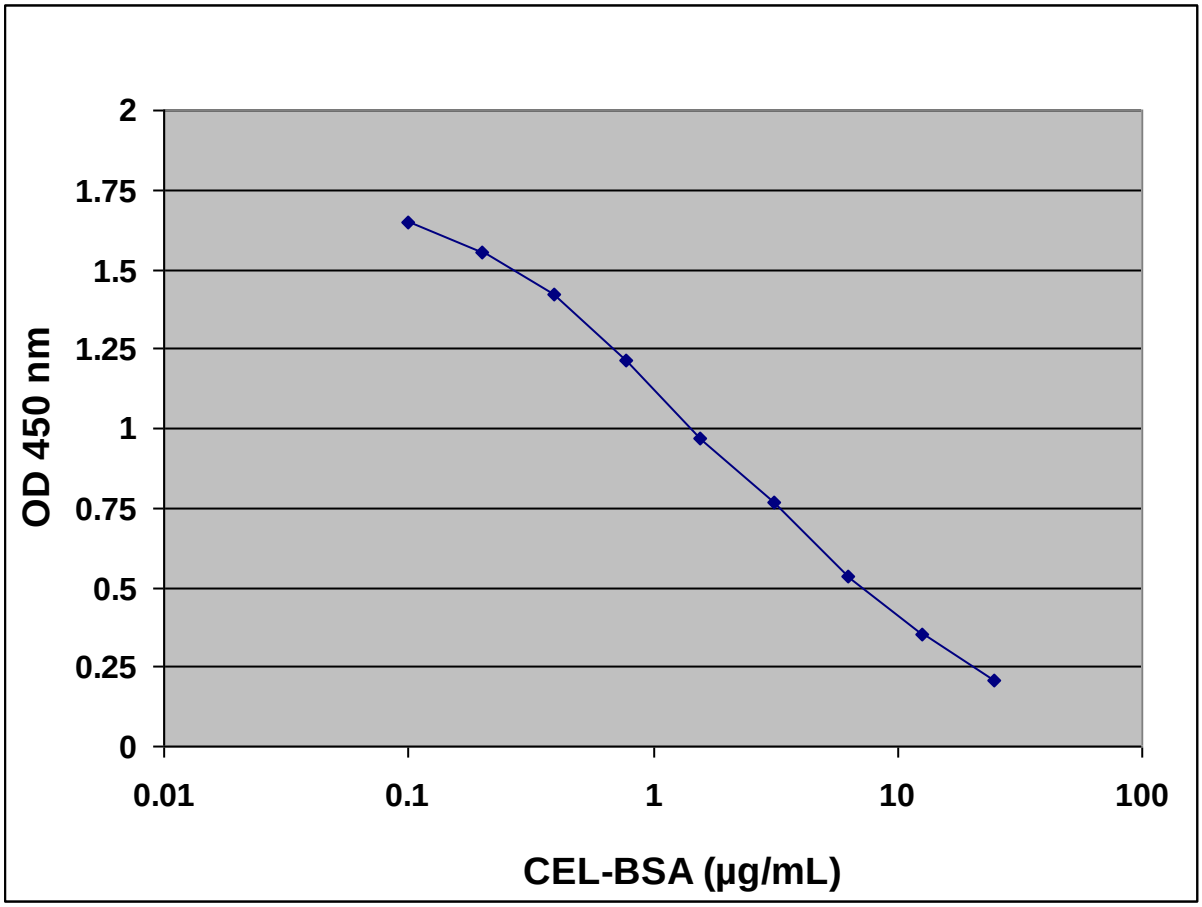

Figure 2: CEL-BSA ELISA Standard Curve.

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Recent Product Citations

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Warranty

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