KAMIYA BIOMEDICAL COMPANY

N^{ε}-(carboxymethyl) lysine (CML) Competitive ELISA

For the detection and quantitation of CML protein adducts

Cat. No. KT-952

For Research Use Only. Not for use in diagnostic procedures.

Product Information

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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) is a major antigenic AGE structure. CML concentration is increased in subjects who have diabetes with complications, including nephropathy, retinopathy, and atherosclerosis. CML is also recognized by receptor for AGE (RAGE), and CML-RAGE interaction activates cell signaling pathways such as NF-κB.

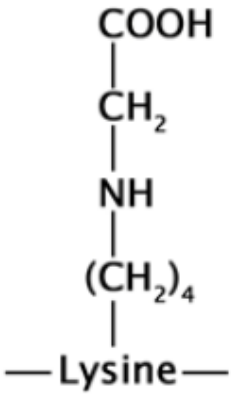

Figure 1. Structure of N^{ε}-(carboxymethyl) lysine (CML)

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

ASSAY PRINCIPLE

First, a CML conjugate is coated on the ELISA plate. The unknown CML protein samples or CML-BSA calibrators are then added to the CML conjugate preabsorbed plate. After a brief incubation, the anti-CML monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The content of CML protein adducts in unknown samples is determined by comparison with the predetermined CML-BSA calibration curve.

COMPONENTS

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate: One strip well 96-well plate.
2. Anti-CML Antibody (1000X): One 10 µL vial of anti-CML antibody.
3. Secondary Antibody, HRP Conjugate (1000X): One 20 µL vial.
4. Assay Diluent: One 50 mL bottle.
5. 10X Wash Buffer: One 100 mL bottle.
6. Substrate Solution: One 12 mL amber bottle.
7. Stop Solution: One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. CML-BSA Calibrator: One 50 µL vial of 1.0 mg/mL CML-BSA in PBS. CML-BSA is prepared as described by Koito *et al*. and it has 15 moles of CML per mole of BSA.
2. 1000X CML Conjugate: One 20 µL vial.
3. 100X Conjugate Diluent: One 300 µL vial.

MATERIALS REQUIRED BUT 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

Aliquot and store the Anti-CML Antibody, CML-BSA Calibrator, CML Conjugate and 100X Conjugate Diluent at -20ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

REAGENT PREPARATION

• CML Conjugate Coated Plate:

Note: The CML 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 1X CML Conjugate by diluting the 1000X CML Conjugate in 1X Conjugate Diluent. Example: Add 5 µL of 1000X CML Conjugate to 4.995 mL of 1X Conjugate Diluent.

3. Add 100 µL of the 1X CML Conjugate to each well to be tested and incubate overnight at 4ºC. Remove the CML 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 Concentrate to 1X with deionized water. Stir to homogeneity.

• Anti-CML Antibody and Secondary Antibody: Immediately before use, dilute the Anti-CML antibody 1:1000 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

CALIBRATOR PREPARATION

Prepare a dilution series of CML-BSA calibrators in the concentration range of 0 to 12.5 µg/mL by diluting the CML-BSA Calibrator in Assay Diluent (Table 1).

Standard Tubes	1 mg/mL CML-BSA Standard (µL)	Assay Diluent (µL)	CML-BSA (µg/mL)	CML (ng/mL)
1	5	395	12.5	576
2	200 of Tube #1	200	6.25	288
3	200 of Tube #2	200	3.13	144
4	200 of Tube #3	200	1.56	72
5	200 of Tube #4	200	0.78	36
6	200 of Tube #5	200	0.39	18
7	200 of Tube #6	200	0.20	9
8	200 of Tube #7	200	0.10	4.5
9	200 of Tube #8	200	0.050	2.25
10	0	200	0	0

Table 1. Preparation of CML-BSA Standards

ASSAY PROCEDURE

1. Prepare and mix all reagents thoroughly before use. Each CML sample including unknown and calibrator should be assayed in duplicate.

2. Add 50 µL of unknown sample or CML-BSA calibrator to the wells of the CML 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-CML 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 2-20 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 CML Competitive ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

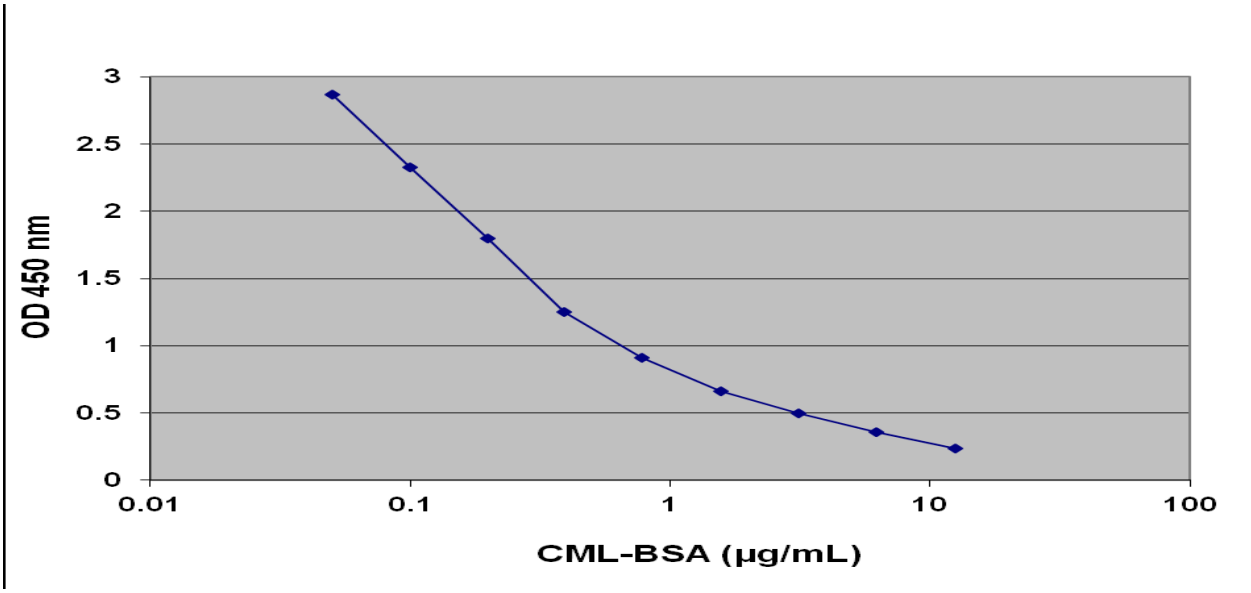

Figure 2: CML-BSA Competitive ELISA Calibration Curve.

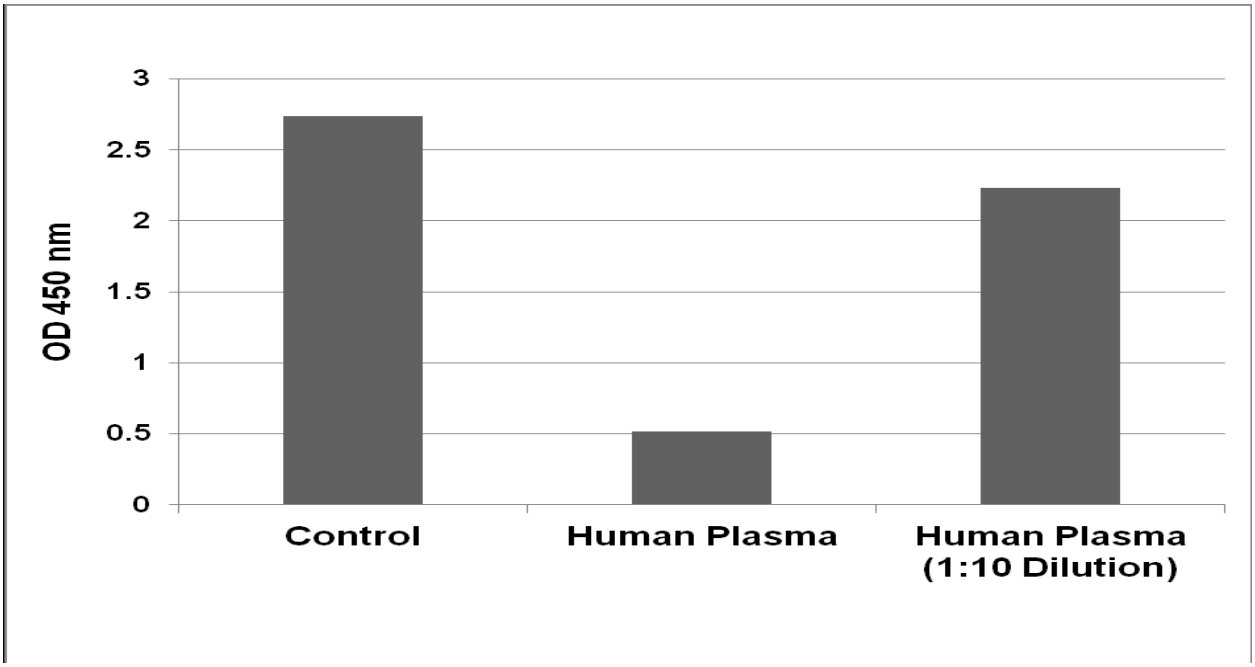

Figure 3: CML Protein Adduct in Human Plasma.

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