Echelon Biosciences Inc.

Advanced Glycation Endproducts (AGEs): Nε-(Carboxymethyl) Lysine (CML) Competitive ELISA II

K-3900s (96 tests)

Support: echelon@echelon-inc.com

Description: Competitive ELISA that quantifies carboxymethyl lysine (CML) in biological samples.

Materials Provided

Catalog #	Description	Quantity
K-3901s	CML Detection Plate	1 Plate
K-3902	CML Standard	1 Vial
K-3903s	CML Detector	1 Bottle
K-SEC1	Secondary Detector	2 Vials
K-DIL1	Assay Diluent	1 Bottle
K-PBST3	10X PBS-T Buffer	1 Bottle
K-TMB1	TMB Solution	1 Bottle
K-STOPt	1N H_2SO_4 Stop Solution	1 Bottle
---	Microtiter plate seal	3 seals

Additional Materials Provided by User

- Microtiter plate reader capable of reading absorbance at 450 nm
- Pipettes (20 µL, 200 µL, and 1,000 µL)
- Reagent grade water
- Plate shaker or similar

Quick Protocol

Bring reagents to room temperature.

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Prepare CML Standards.

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Prepare samples and CML Detector with Assay Diluent (K-DIL1)

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Add standards, samples, and CML Detector to plate. Incubate for 1 hour at room temperature.

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Discard the solutions and wash plate 3 times.

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Prepare Secondary Detector and add to plate. Incubate for 30 minutes at room temperature.

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Discard the solutions and wash plate 3 times.

↓

Add TMB solution to the plate. Cover and incubate for 30 minutes at room temperature.

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Read absorbance at 450 nm.

Storage

Upon receipt store kit Part 1 at -20 °C and Part 2 at 4 °C. Under proper storage conditions, the kit components are stable for 6 months from date of receipt.

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Background

Carboxymethyl Lysine (CML) is an advanced glycation endproduct (AGE) formed by oxidative stress and glycosylation. AGEs, including CML, can accumulate in tissues causing inflammation. Excessive AGEs also reduce antioxidant defense, weaken immune systems, impair DNA repair mechanisms, and promote the accumulation of toxins and infections in tissues[1]. CML adducts have been found to accumulate in many diseases including Alzheimer's disease[2], diabetes[3], chronic obstructive pulmonary disease (COPD)[4], accelerated periodontal disease[5], renal disease[6] and atherosclerosis[7].

Assay Design

Echelon's CML Competitive ELISA II is a second generation competitive immunoassay that quantifies CML in biological samples. CML within the sample competes with plate-bound CML for detector binding. A decrease in signal indicates an increase in CML concentration within the sample.

Assay Notes

1. All steps of the procedure must be followed as described. Failure to do so will affect assay performance.
2. The assay can be run multiple times within a 1 week period as long as the reagents are stored as instructed. All reagents must be brought to room temperature prior to use.

Sample Preparation

1. Samples should be free of any debris that may interfere with the assay.
2. A sample dilution of 1:5 is recommended using the assay Diluent. Additional or fewer dilutions can be made depending on sample type and concentration. A 1:2 dilution is the minimum dilution suggested.
3. Pre-dilution can be done if necessary using PBS or similar. Final sample dilutions must be made with the assay Diluent.
4. Sample buffers/diluents, especially lysis or homogenate buffers should be run as controls in the assay to determine any interference.
5. When analyzing biological samples, we advise running a known normal (low) CML sample and a disease (high) CML sample in conjunction with your unknown samples. These will serve as positive and negative controls to aid in distinguishing between normal healthy samples and disease samples.
6. The CML assay has been tested using human serum, plasma and urine samples. Other sample types or species sources can be tested but may require optimization.

Assay Procedure

Please read through the entire protocol along with the "Sample Preparation" and "Assay Notes" sections prior to beginning the assay.

1. Bring assay kit to room temperature before use.
2. Prepare 1X PBS-T by diluting the 30 mL of 10X PBS-T Buffer (K-PBST3) with 270 mL reagent grade water. Store at 4°C when not in use. Buffer is stable for 6 months after preparation.
3. The Assay Diluent (K-DIL1) comes ready to use. Store at 4°C when not in use.
4. Prepare the CML standard curve from the 10,000 ng/mL stock solution (K-3902) according to Table 1 below. Store the 10,000 ng/mL CML Standard at -20°C when not in use. An example standard curve is shown in Figure 1.

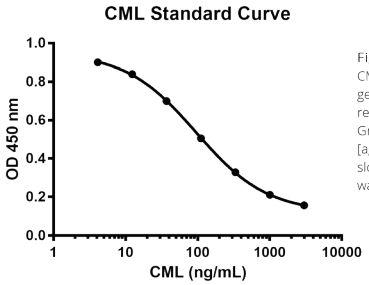

Figure 1. CML standard curve was generated using non-linear regression analysis with Graphpad software. A log [agonist] vs. response-variable slope (four parameter) analysis was used.

5. Prepare samples (see "Sample Preparation" section) by diluting with Assay Diluent. The Assay Diluent (K-DIL1) comes ready to use. Store at 4°C when not in use. It is recommended that samples be run in duplicate according to the plate layout below. Samples should be diluted just prior to use.
6. Prepare the CML Detector working solution by adding 6 mL of Assay Diluent to the bottle of CML Detector (K-3903s). Mix gently to fully resuspend the detector. Store diluted CML Detector at 4°C when not in use. Reagent is stable for one week at 4°C.
7. Add CML standards, samples and detector to the CML Detection Plate (K-3901s). See Table 2 for the plate layout.
   a. Add 50 µL of the CML standards (step 4) to the CML Detection Plate.
   b. Add 50 µL of the diluted samples (step 5), in duplicate, to the CML Detection Plate.
   c. Optional: A "Blank" control can be run by adding 100 µL of Assay Diluent to 1-2 of the sample wells.
   d. Add 50 µL of the CML Detector working solution to each standard and sample well (do not add CML Detector to the "Blank" wells if applicable.)
8. Cover plate with a plate seal and incubate at room temperature for 1 hour with gentle agitation on a plate shaker. Unused portion of the CML Detection Plate can be stored at 4°C covered with a plate seal.
9. After the incubation, discard solution from the plate and wash 3X with 200 µL 1X PBS-T buffer (step 2) per well.
10. Prepare the Secondary Detector working solution by diluting the Secondary Detector stock (K-SEC1) 1:25 in 1X PBS-T. Note: the Secondary Detector stock (K-SEC1) comes in two vials with 300 µL each. Mix gently to fully resuspend the detector. Prepare only what is needed for the portion of the plate being utilized. 12 mL is

Table 1, CML Standards

CML Standard Concentration	CML Stock (K-3902) or Previous Dilution	Assay Diluent (K-DIL1)
3,000 ng/mL	90 µL of 10,000 ng/mL Stock Solution	210 µL
1,000 ng/mL	100 µL of 3,000 ng/mL Solution	200 µL
333 ng/mL	100 µL of the 1,000 ng/mL Solution	200 µL
110 ng/mL	100 µL of the 333 ng/mL Solution	200 µL
37 ng/mL	100 µL of the 110 ng/mL Solution	200 µL
12 ng/mL	100 µL of the 37 ng/mL Solution	200 µL
4 ng/mL	100 µL of the 12 ng/mL Solution	200 µL
0 ng/mL	None	200 µL

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Table 2, Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	3,000 ng/mL	3,000 ng/mL	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17	Sample 25	Sample 25	Sample 33	Sample 33
B	1,000 ng/mL	1,000 ng/mL	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18	Sample 26	Sample 26	Sample 34	Sample 34
C	333 ng/mL	333 ng/mL	Sample 3	Sample 3	Sample 11	Sample 11	Sample 19	Sample 19	Sample 27	Sample 27	Sample 35	Sample 35
D	110 ng/mL	110 ng/mL	Sample 4	Sample 4	Sample 12	Sample 12	Sample 20	Sample 20	Sample 28	Sample 28	Sample 36	Sample 36
E	37 ng/mL	37 ng/mL	Sample 5	Sample 5	Sample 13	Sample 13	Sample 21	Sample 21	Sample 29	Sample 29	Sample 37	Sample 37
F	12 ng/mL	12 ng/mL	Sample 6	Sample 6	Sample 14	Sample 14	Sample 22	Sample 22	Sample 30	Sample 30	Sample 38	Sample 38
G	4 ng/mL	4 ng/mL	Sample 7	Sample 7	Sample 15	Sample 15	Sample 23	Sample 23	Sample 31	Sample 31	Sample 39	Sample 39
H	0 ng/mL	0 ng/mL	Sample 8	Sample 8	Sample 16	Sample 16	Sample 24	Sample 24	Sample 32	Sample 32	Sample 40	Sample 40

sufficient for the entire plate. Store Secondary Detector stock at 4°C when not in use. Add 100 µL of the diluted Secondary Detector to each well of the CML Detection plate. Cover plate with a plate seal and incubate at room temperature for 30 minutes with gentle agitation on a plate shaker.

11. After the incubation, discard solution from the plate and wash 3X with 200 µL 1X PBS-T buffer (step 2) per well.
12. Add 100 µL of the TMB solution (K-TMB1) to each well of the CML Detection plate. Let blue color develop for 30 minutes in a dark location. Store TMB solution at 4°C when not in use.
13. Add 50 µL of the 1 N H_2SO_4 Stop Solution (K-STOPt) to each well to stop the reaction. Tap plate to mix. Store 1 N H_2SO_4 Stop Solution at room temperature when not in use.
14. Read absorbance at 450 nm.
15. Generate a best fit curve of the CML standards in order to interpolate sample values (see figure 1).

References (Background)

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References (Product Publications)

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