- Aging/Glycation Assay Kit series -

Collagen AGEs Assay Kit, CML-Specific, Glyoxal

Cat. AAS-AGE-K02E

Introduction

Although carbohydrates are indispensable for ATP production, excess amounts of carbohydrates modify amino residues of amino acids such as lysine and arginine, and results in the irreversible functional disorders of proteins by changing the three-dimensional structure and net negative charge in patients with disordered metabolism. Since this reaction was first reported by Louis Camille Maillard in 1912, the reaction is called the Maillard reaction, or glycation. The Maillard reaction is divided by early and advanced stages. Early stage generates Amadori rearrangement products, such as haemoglobin A1c, whereas advanced stage generates the AGEs (advanced glycation end products), which is characterized by colour in brown and protein cross-linking. Collagen, the structural protein that forms skin, blood vessel wall and bone, also undergo glycation reaction.

CML is generated by the oxidative cleavage of Amadori products by hydroxyl radicals (1), peroxynitrite (2) and hypochloric acid (3), thus suggesting that CML is an important biological marker of oxidative stress *in vivo*. CML concentration, adjusted for age and duration of diabetes, is further increased in patients who have severe complications, including nephropathy (4), retinopathy, and atherosclerosis.

CML ELISA Assay Kit provides rapid detection of CML formed by glycation with glyoxal on the collagen coating plate. This kit is suitable to the research for functional foods and cosmetic materials which have anti-glycation activity.

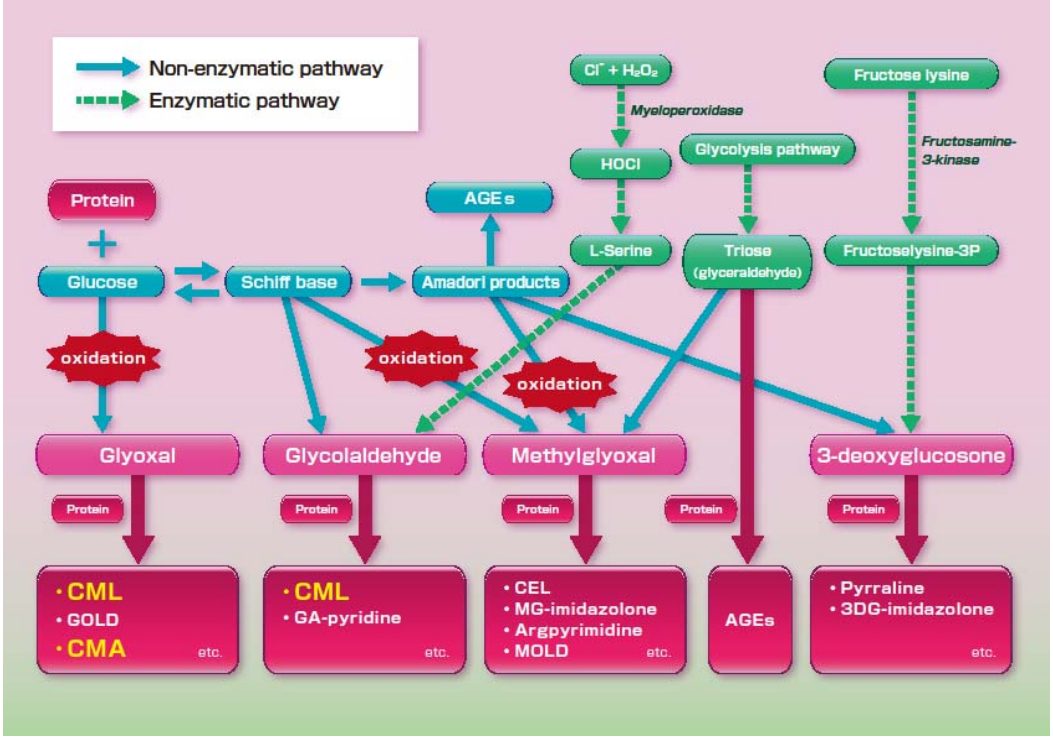


Fig. 1. Production pathway of AGEs generating aldehyde

《I-1. Kit Components》

Components	quantity	Storage
96-well Collagen coated plate	One strip-well plate	4 °C
Microplate seal	2 sheets	
Anti-CML Antibody（100X）	100 uL	
Blocking Buffer	50 mL	
HRP Conjugate Secondary Antibody（100X）	100 µL	
Sample Dilution Buffer	30 mL	
Washing Buffer（10X）	50 mL	
Substrate Solution	10 mL	
Stop Solution	10 mL	
Glyoxal Solution (20 mM)	5 mL	
Aminoguanidin Solution (10 mM) ※ positive control	250 µL	

※ **The kit provides sufficient reagents to perform up to 96 assays.**

Store the 96-well collagen coated plate at -20 °C for the long term storage.

《I-2. Assay principle》

Collagen coated on 96-well plate is glycated by glyoxal and CML is formed onto collagen coating wells for 24hrs at 37 °C. The inhibitory effects of glycation by aminoguanidin (positive control) or samples are probed with an anti-CML antibody, followed by an HRP conjugated secondary antibody. The inhibitory effect of glycation in unknown sample is determined by comparing with the inhibitory effect of glycation by aminoguanidin (positive control).

"Collagen AGEs Assay Kit, CML-Specific, Glyoxal" is suitable to the research for functional foods and cosmetic materials which have an anti-glycation activity.

《II. Assay protocol》

Materials Not Supplied

- Purified water
- 10 µL～1000 µL micropipette
- 50 µL～200 µL multi-channel micropipette
- Reservoir
- Microplate reader（wavelength 492 nm）
- 37 °C incubator（under humid condition）

Preparation of Regents

1X Washing Buffer

- Dilute the 10X Washing Buffer concentrate to 1X with purified water.

0.4 mM, 2 mM Aminoguanidine (positive control) Solutions

- Dilute the 10 mM Aminoguanidine Solution concentrate to 0.4 mM, 2 mM with Sample Dilution Buffer.

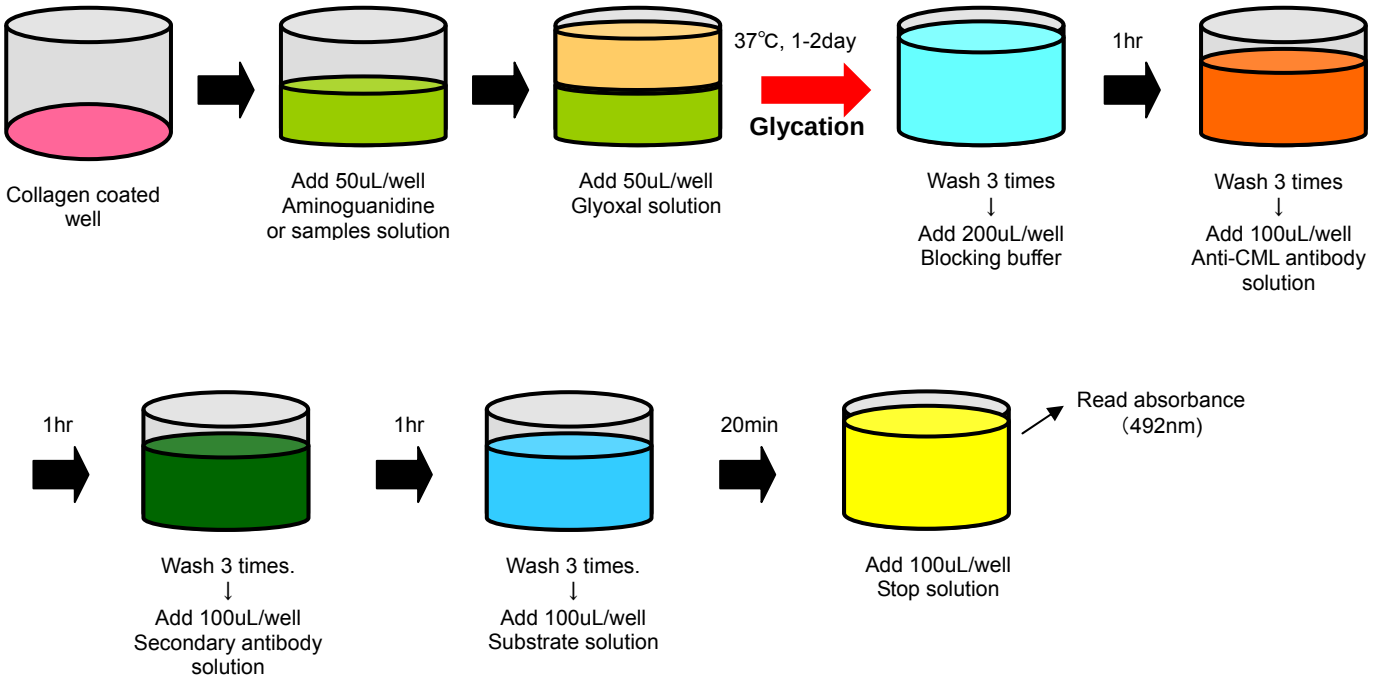
Sample Solutions

- Dilute each sample solution with Sample Dilution Buffer and filtrate them with 0.2 µm filter.

Anti-CML Antibody and Secondary Antibody（prepared at time of use）

- Immediately before use, dilute the Anti-CML antibody 1:100 and Secondary Antibody 1:100 with Blocking Buffer. Diluted antibody solution can be stored for 1week at 4 °C.

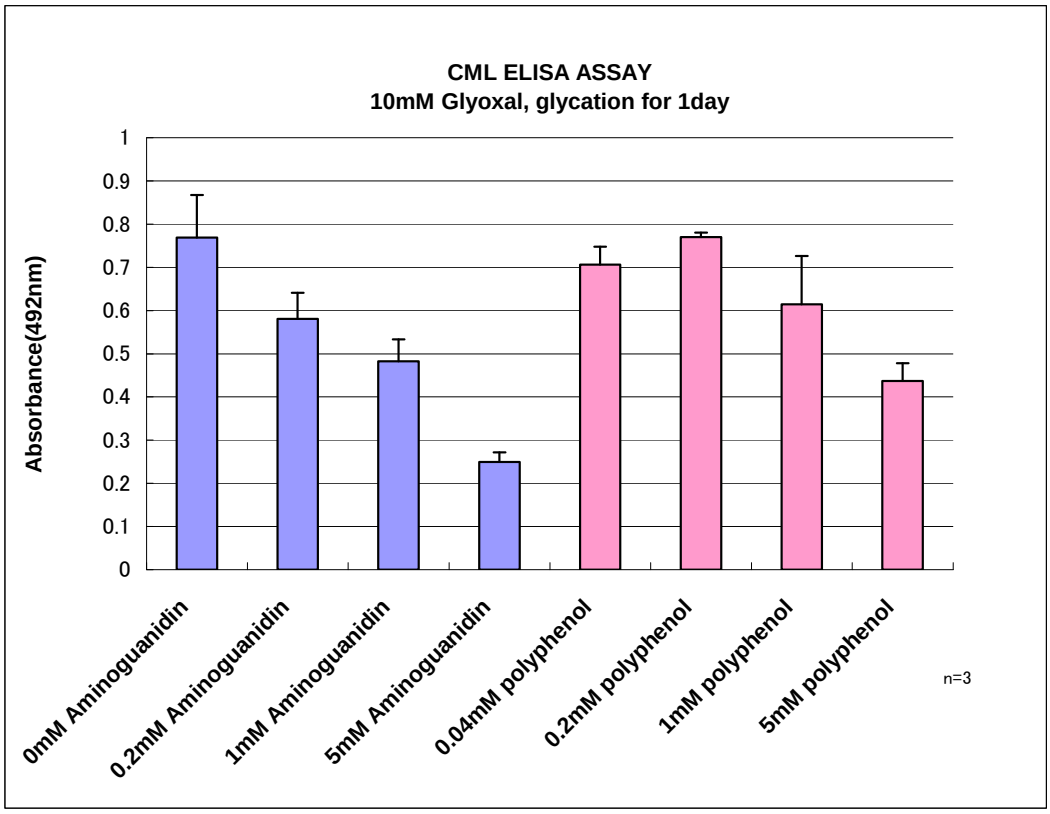
ELISA Assay Protocol



1. The collagen coated plate goes back up to room temperature before use. Take a necessary number of well out of the aluminum pouch. Please cut a seal at suitable size.
2. Add 50 µL per well of the 0, 0.4, 2, 10 mM Aminoguanidin Solution(positive control) or sample solutions to the 96-well collagen coated plate.
3. Add 50 µL/well of Glyoxal Solution.
4. Seal the plate well with the microplate seal and incubate under humid condition for 24-48hrs at 37 °C to avoid drying the well up (but do not use CO_2 incubator).
5. Wash wells 3 times with 200 µL/well of Washing Buffer. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Washing Buffer.
6. Add 200 µL/well of the Blocking Buffer and incubate for 1hour at room temperature.
7. Remove the Blocking Buffer from each well completely and wash wells 3 times with 200 µL/well of Washing Buffer. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Washing Buffer. Add 100 µL/well of the Anti-CML Antibody Solution and incubate for 1hour at room temperature.
8. Remove the Anti-CML Antibody Solution from each well completely and wash 3 times with 200 µL/well of Washing Buffer. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Washing Buffer. Add 100 µL/well of the Secondary Antibody Solution and incubate for 1 hour at room temperature.
9. Remove the Secondary Antibody Solution from each well completely and wash 3 times with 200 µL/well of Washing Buffer. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Washing Buffer. Warm the substrate solution to room temperature. Add 100 µL/well of the Substrate solution and incubate at room temperature for 20 minute.
10. Confirm the color and stop the enzyme reaction by additing 100 µL of Stop Solution to each well.
11. Absorbance of each well should be read with the microplate reader using 492 nm.

《III. Example of Results》

The following figure shows the inhibitory effect of aminoguanidine and phytochemicals on the CML formation.



《IV. References》

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4. Mera K., Nagai M., Brock JW., Fujiwara Y., Imai H., Murata T., Maruyama T., Baynes JW., Otagiri M., Nagai R. Glutaraldehyde is an Effective Cross-linker for Production of Antibodies Against Advanced Glycation End Products. J. Immunol. Methods 334 (1-2), 82-90 (2008)

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