COSMO BIO CO., LTD.
Inspiration for Life Science

Instruction manual

Collagen Glycation Assay Kit, Glucose / Fructose

Catalog Number : AK70-COS (192 tests)

For research use only, Not for diagnostic use.

- Please read this manual thoroughly before use –

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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. This reaction is initiated by the nonenzymatic reaction of reducing sugars with free amino groups on proteins to form Amadori products. The Amadori products undergo a variety of irreversible dehydration and rearrangement reactions, leading to the formation of advanced glycation end products (AGEs). AGEs have adverse effects on the functional properties of proteins. Many AGEs have fluorescent and covalent cross-linking properties. The accumulation of AGEs is thought to play an important role in the pathogenesis of diabetic patients and the aging process. The collagen that forms bone, skin, and blood vessel is also glycated.

The Collagen Glycation Assay Kit, Glucose / Fructose can detect the fluorescent AGEs produced by the glycation reaction between collagen and sugar on an ongoing basis. The inhibitory effect of samples on the glycation of collagen can be assayed in a 96-well plate. This kit provides sufficient reagents to perform up to 192 assays.

This kit tests the ability to inhibit AGE formation and would be useful for checking usefulness of functional foods or cosmetic materials.

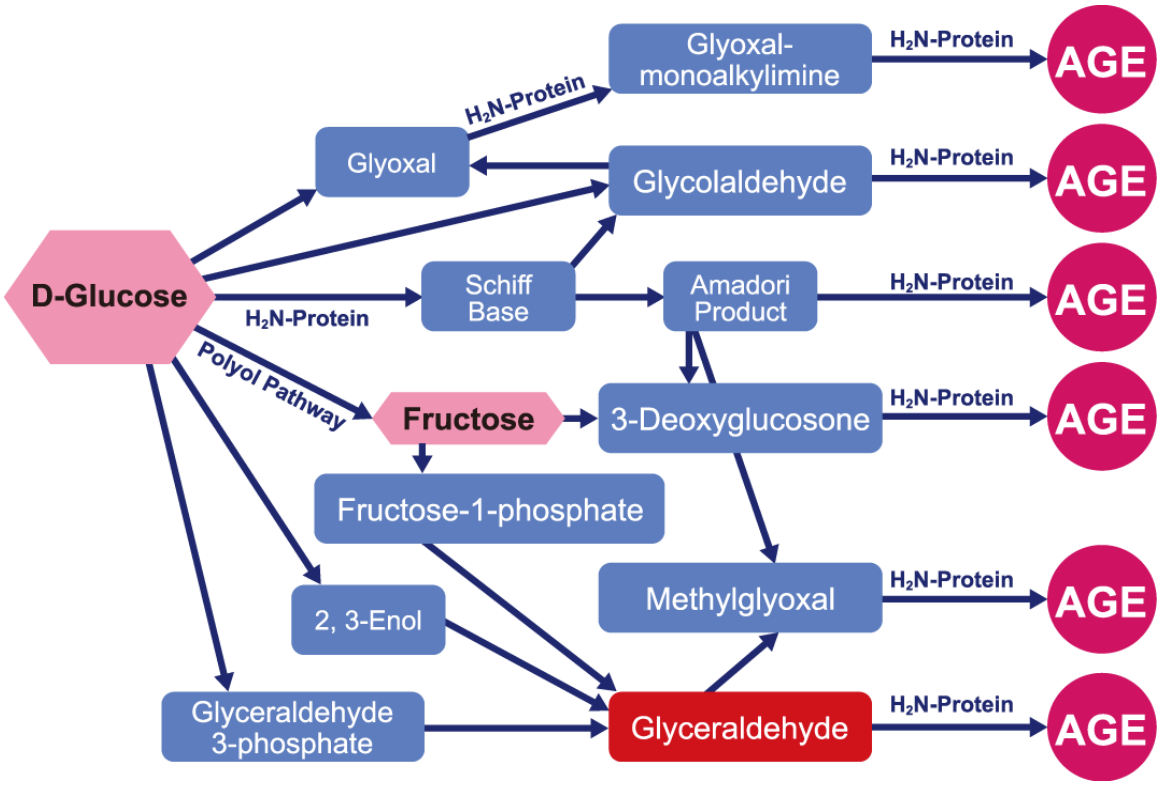


Figure 1. Possible routes for the formation of advanced glycation end-products (AGEs)

《 Assay principle 》

The collagen gel formed in 96-well plates generates fluorescence after long-term incubation with glucose or fructose at 37°C. Some reagents or natural products inhibit this reaction. The Collagen Glycation Assay Kit, Glucose / Fructose is a complete assay system, which is designed to measure fluorescent AGEs using a fluorescence micro plate reader equipped with a 370 nm excitation filter and a 440 nm emission filter.

《I. Kit components 》

Components	Quantity	Storage
Collagen Solution	10 mL	4°C
Glucose Solution (200 mM)	10 mL	
Fructose Solution (200 mM)	10 mL	
Sample Dilution Buffer	30 mL	
Aminoguanidine Solution (20 mM): Positive Control	0.5 mL	

* One kit contains sufficient reagents for 192 assays (tubes or 96-well plates)
* Additional materials required:
  - 96-well black plate (sterile, clear bottom)
    Greiner [μCLEAR-PLATE BLACK Cat. No. 655090] is recommended.
  - Fluorescent microplate reader
    (Mode: fluorescence bottom reading, excitation wavelength: 370 nm, emission wavelength: 440 nm)

《II. Assay protocol: 96-well plate》

(1) Collagen solution is stored on ice (<10°C) before testing.

(2) Add 50 μL of the collagen solution to the 96-well black plate.

Incubate the plate for 18–24 h at 37°C with high humidity to avoid the wells drying up (do not use a CO_2 incubator). The collagen solution changes into a white gel.

(3) Prepare the positive control by diluting the 20 mM aminoguanidine solution to concentrations of 0, 0.8, and 4 mM with sample dilution buffer.

(4) Dissolve the samples with the sample dilution buffer and filter through a 0.22-μm filter. Add **10 μL** of the 0, 0.8, 4, and 20 mM aminoguanidine solutions (positive control) or samples to each well.

(5) Add **50 μL** of (i) sample dilution buffer, (ii) 200 mM glucose, or 200 mM fructose solution to each well. Mix thoroughly.

(6) Immediately, start reading the standard and sample wells using a fluorescent microplate reader at an excitation wavelength of 370 nm and an emission wavelength of 440 nm by fluorescence bottom reading. Record this fluorescence intensity before incubation **(0 week:A).**

(7) Incubate the plate for **1, 2, 3, and 4 weeks (10–30 days)** at 37°C in high humidity conditions to avoid the wells drying up (do not use a CO_2 incubator).

(8) Read the fluorescent intensity after **1, 2, 3, and 4 weeks (10–30 days:B)** with a fluorescent microplate reader at 37°C.

(9) The inhibitory effects of the samples on glycation are calculated as follows.

Fluorescent intensity B (1,2,3 and 4 weeks) − Fluorescent intensity A (0 week) = Inhibitory effect of glycation

《III. Assay Example 》

Figure 2.

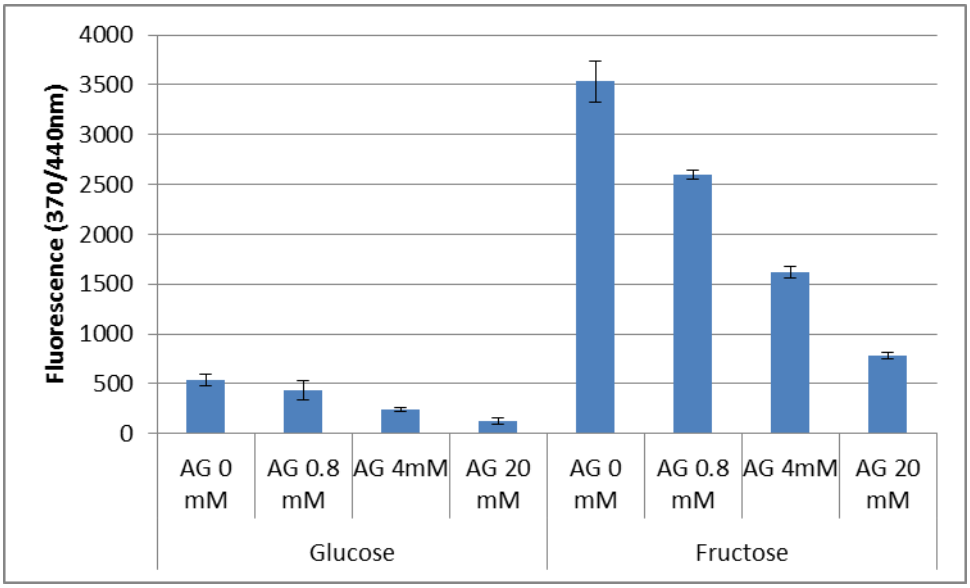


Figure 3. Time course of the glycation of the collagen gel after the addition of fructose and the inhibitory effects of aminoguanidine. The formation of AGEs with fructose was measured on the basis of the fluorescence after incubation at 37°C for 1, 2, 3, 4, and 5 weeks.

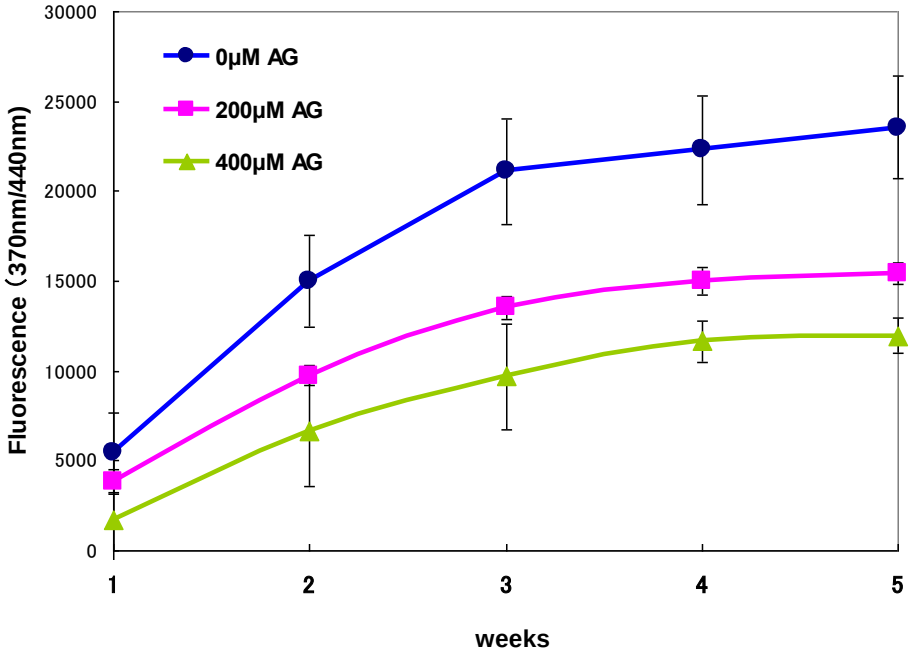
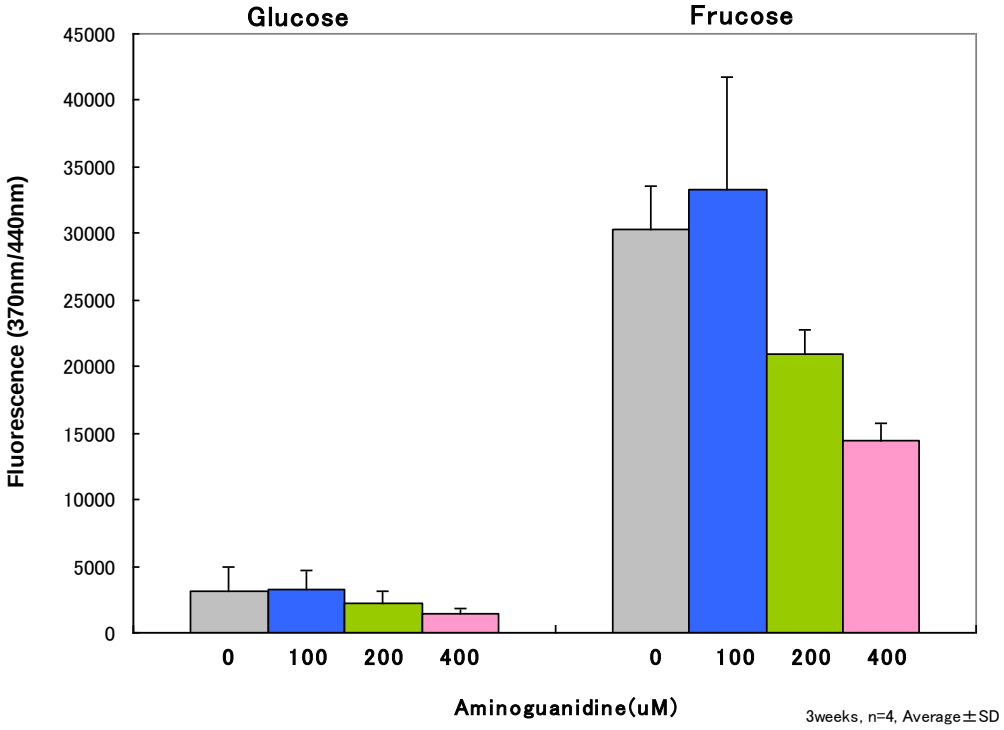


Figure 4. Effects of glucose or fructose on the glycation of collagen with glyceraldehyde and the inhibitory effects of aminoguanidine. The formation of AGEs was measured on the basis of the fluorescence after incubation at 37°C for 3 weeks.



《IV. References》

(1) A. Nishikawa, T. Taira, K. Yoshizato. *In Vitro* Maturation of Collagen Fibrils Modulates Spreading, DNA Synthesis, and Collagenolysis of Epidermal Cells and Fibroblasts. Exp. Cell Res. (1987) 171, 164–177.

(2) H. Shoda et al. Inhibitory Effects of Tenilsetam on the Maillard Reaction. Endocrinology (1997) 138, 1886–1892.

(3) J. Takino et al. The Formation of Intracellular Glyceraldehyde-Derived Advanced glycation End-Products and Cytotoxicity. J Gastroenterol. (2010) 45, 646–655 PMID: 20084527.

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