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Inspiration for Life Science

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Collagen Glycation Assay Kit, Glyceraldehyde

Catalog No. AK71(Japan)
PMC-AK71 (Outside Japan)

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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.

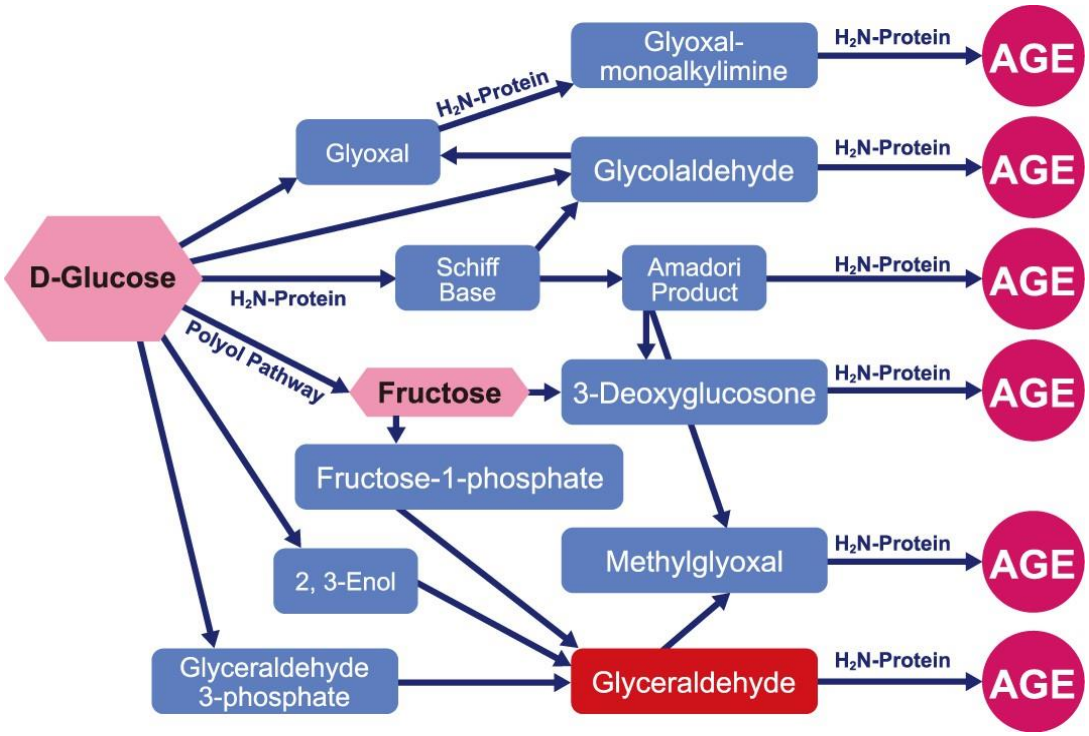


Figure 1. Possible routes for the formation of advanced glycation end-products (AGEs)

《 Assay principle 》

Collagen Glycation Assay Kit, Glyceraldehyde is a complete assay system designed to measure the fluorescent AGEs using the fluorescence microplate reader equipped with a 370nm excitation filter and 440nm emission filter.

《Ⅰ. Kit components 》

Components	Quantity	Storage
Collagen Solution	10 mL	4℃
Glyceraldehyde Solution（500mM）	2 mL	
Sample Dilution Buffer	30 mL	
Aminoguanidine Solution（20mM）: Positive control	0.5 mL	

* One kit contains regents for 192 assays (96 well Plate)
* Additional materials required
  - 96well black plate（clear bottom、sterile）
    Greiner [μCLEAR－PLATE BLACK Cat.No.655090] is recommended.
  - Fluorescent microplate reader
    (Mode: Fluorescence Bottom Reading, Excitation Wavelength: 370nm, Emission Wavelength: 440nm）

《Ⅱ. Assay protocol－96-well plate－》

(1) Collagen solution is stored into the ice (<10 ℃) before testing.
(2) Add 50 uL of the collagen solution into the 96-well black plate.
Incubate the plate for 18-24hrs at 37℃ under the high humidity condition to avoid drying the well up (but do not use CO_2 incubator). Collagen solution changes into the white gel.
(3) Prepared positive control by diluting the 20 mM Aminoguanidine solution at the concentration of 0, 0.8, 4mM with sample dilution buffer.
(4) Dissolve the samples with the sample dilution buffer and filtrate with 0.22 μm.
Add 40 uL of the positive control or samples to each well.
(5) Add 10uL of 500 mM Glyceraldehyde Solution to each well. Mix thoroughly.
(6) Immediately begin reading standard and sample wells with a fluorescent microplate reader with the Excitation wavelength of 370 nm and an emission wavelength of 440 nm by fluorescence bottom reading.
Peg this fluorescence intensity at before incubation (0 hr) and describe "Fluorescent intensity A".**
(7) Incubate the plate for 24hrs at 37 ℃ under the high humidity condition to avoid drying the well up (but do not
use CO_2 incubator).

After 16–24 hrs, read the fluorescent intensity with a fluorescent microplate reader at 37 ℃.

Peg the fluorescence intensity at after incubation for 24 hrs fluorescent intensity and describe "Fluorescent intensity B".

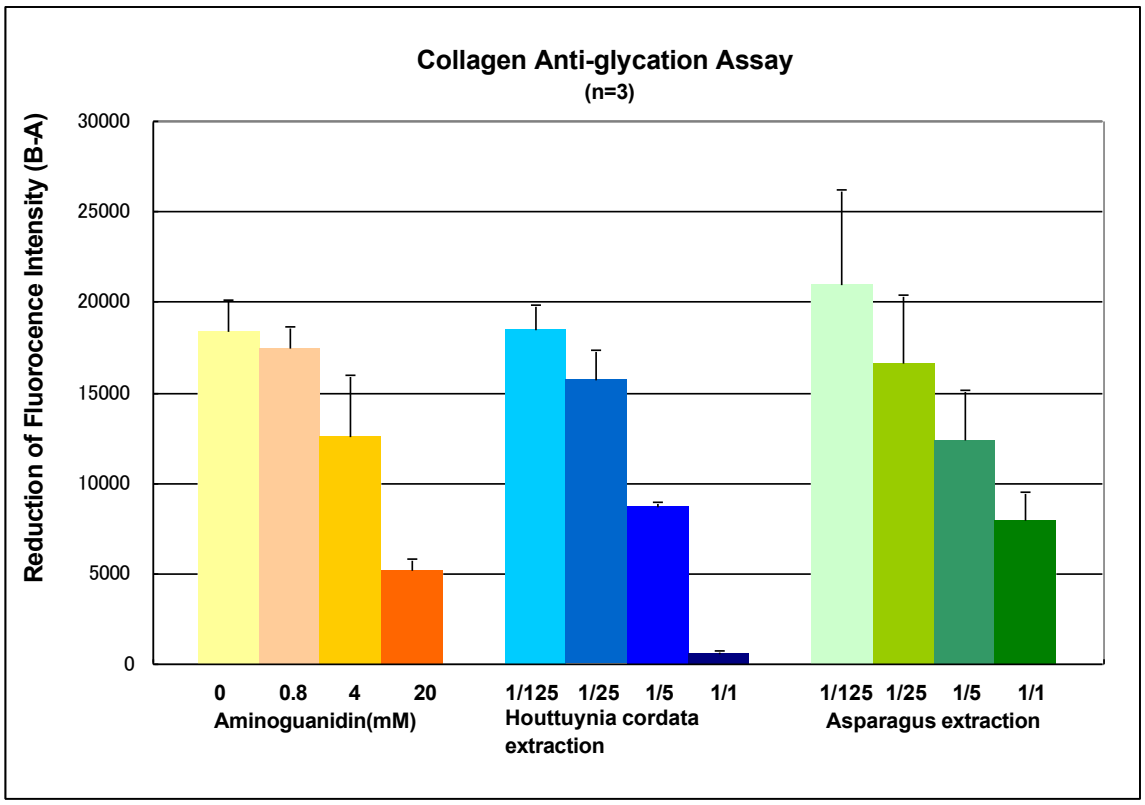
(8) The reduction of fluorescence intensity (Fluorescent intensity B－Fluorescent intensity A) from control

fluorescence intensity is the inhibitory effect of glycation.

** In case samples contain florescent material, subtract the florescence intensity of the sample group without addition of glyceraldehyde (as "sample blank") from the group with glyceral dehyde.

《Ⅲ. Example of Results 》

The following figure demonstrates Collagen Glycation Assay Kit, Glyceraldehyde results that inhibitory effects of aminoguanidin, Houttuynia cordata extraction, and Asparagus extraction on collagen glycation.



《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.

COSMO BIO CO., LTD.

【JAPAN】
TOYO EKIMAE BLDG. 2-20, TOYO 2-CHOME,
KOTO-KU. TOKYO 135-0016, JAPAN
Phone: +81-3-5632-9610
FAX: +81-3-5632-9619
URL: https://www.cosmobio.co.jp/

COSMO BIO USA

【Outside Japan】
2792 Loker Ave West, Suite 101
Carlsbad, CA 92010, USA
e-mail: support@cosmobiousa.com
Phone/FAX: (+1) 760-431-4600
URL: www.cosmobiousa.com