COSMO BIO CO., LTD.
Inspiration for Life Science

Instruction manual

Collagen Glycation Assay Kit, Glyceraldehyde

Catalog No.: PMC-AK71-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 or arginine 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. Many AGEs have fluorescent and convalent cross-linking properties.
Accumulation of AGEs has been thought to play an important role in the pathogenesis of diabetic patients as well as the aging process.

Recent studies have suggested that AGEs can arise not only from sugars but also from carbonyl compounds derived from the autoxidation of sugars and other metabolic pathways. Among different AGEs, there is evidence that glyceraldehyde -derived AGEs are associated with such cytotoxcity.

Collagen Glycation Assay Kit, Glyceraldehyde provides rapid detection of fluorescent AGEs and inhibition assay for glycation of collagen by glyceraldehyde. 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.

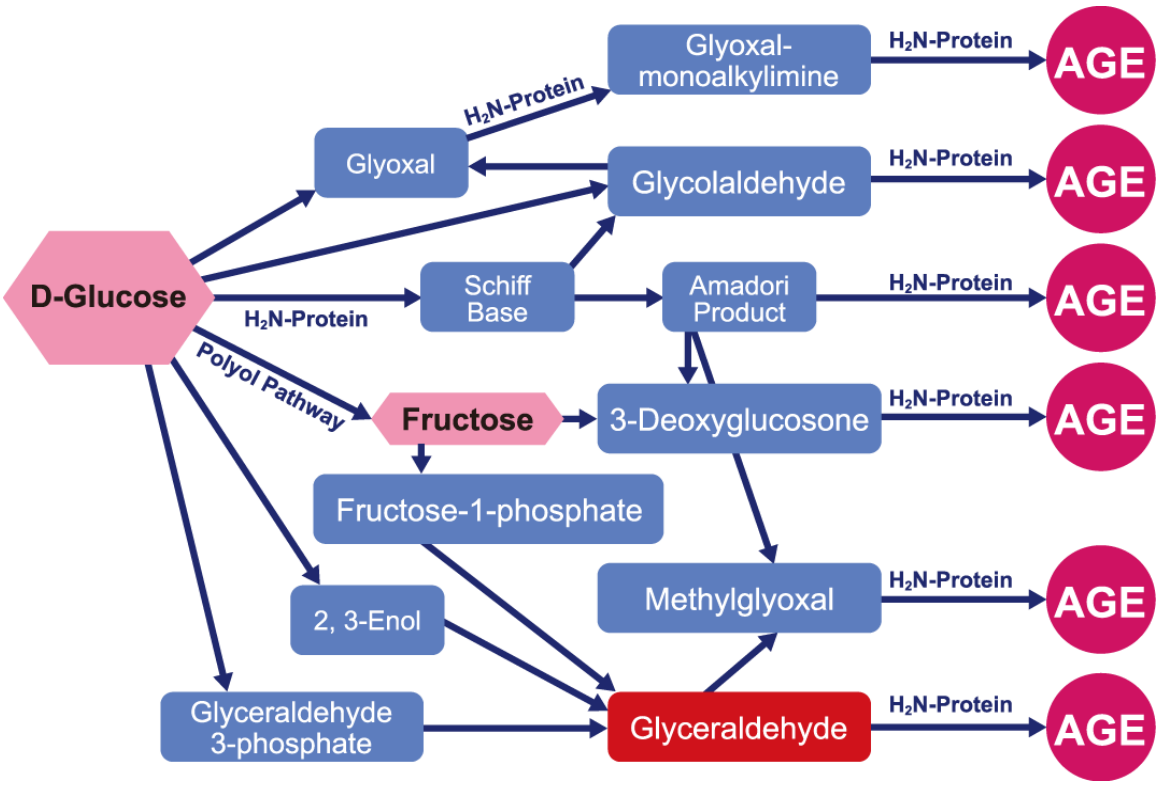


Figure 1. Possible routes of the advanced glycation end-products (AGEs) formation

《 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 Acidic Solution	5 mL	4℃
Neutralizing Solution	5 mL	
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 acidic solution and neutralizing solution are stored into the ice (<10 ℃) before testing.
Add 5ml of neutralizing solution into the bottle of collagen solution and pipette up and down several times to completely mixing gently to avoid bubbling at <10℃.

(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).

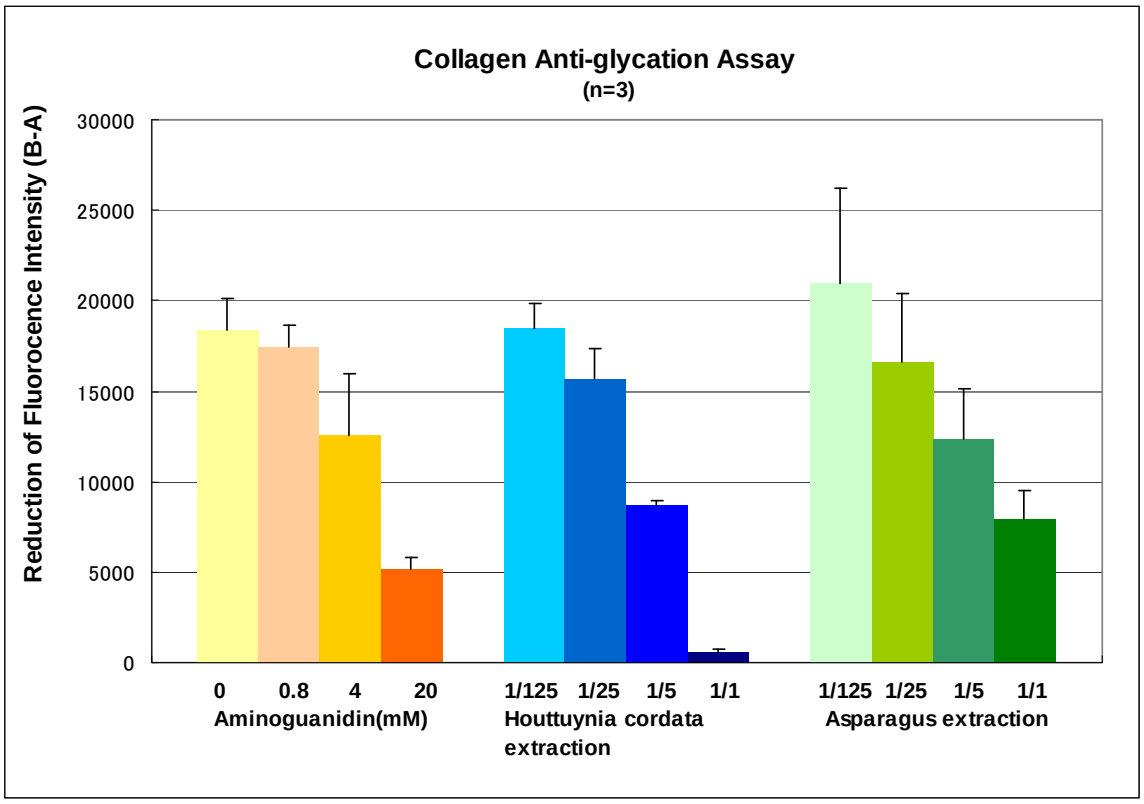
(8) After 24 hrs, read the fluorescent intensity with a fluorescent microplate reader at 37 ℃.
Peg the fluorescence intensity at after incubation for 24 hrs fluorescent intensity and describe "Fluorescent intensity B".

(9) 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 glyceraldehyde.

《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, p164-177.

(2) H. Shoda et al. Inhibitory Effects of Tenilsetam on the Maillard Reaction. Endocrinology (1997) 138, p1886-1892.

(3) Jun-ichi Takino et al. The formation of intracellular glyceraldehyde-derived advanced glycation end-products and cytotoxicity. J Gastroenterol. 2010 Jun;45(6):646-55. PMID: 20084527

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