COSMO BIO CO., LTD.
Inspiration for Life Science

Instruction manual

Albumin Glycation Assay Kit
[Glyceraldehyde]

Catalog Number : AAS-AGE-K01E(96*2 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. 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.

Albumin Glycation Assay Kit, Glyceraldehyde provides rapid detection of fluorescent AGEs and inhibition assay for glycation of albumin solution by glyceraldehyde. This kit provides sufficient reagents to perform up to 192 assays, and tests the ability to inhibit AGE formation and would be useful for checking usefulness of functional foods or cosmetic materials.

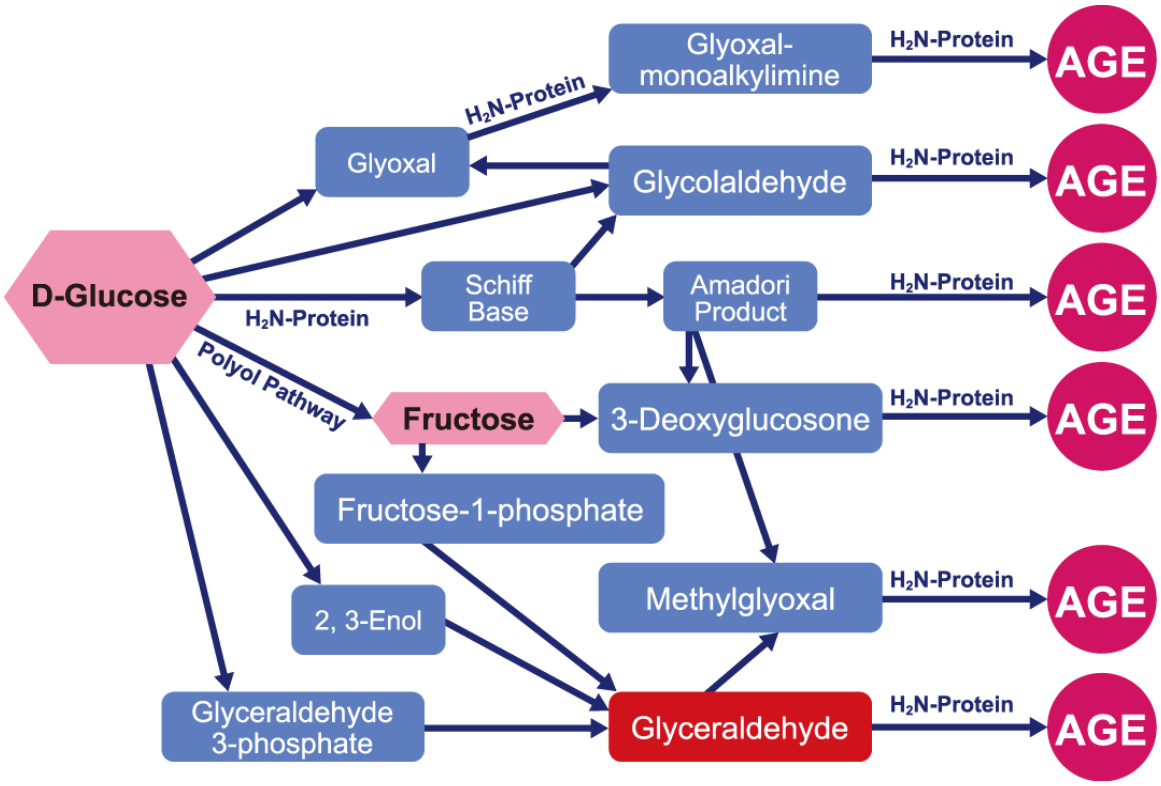


Figure 1. Possible routes of the advanced glycation end-products (AGEs) formation

《 Assay principle 》

Albumin 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
Bovine Serum Albumin（BSA）Solution	10 mL	4℃
Glyceraldehyde Solution（500mM）	2 mL	
Dilution Buffer	30 mL	
Aminoguanidine Solution（20mM）: Glycation Standard	0.5 mL	

* One kit contains regents for 192 assays (Tube or 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）

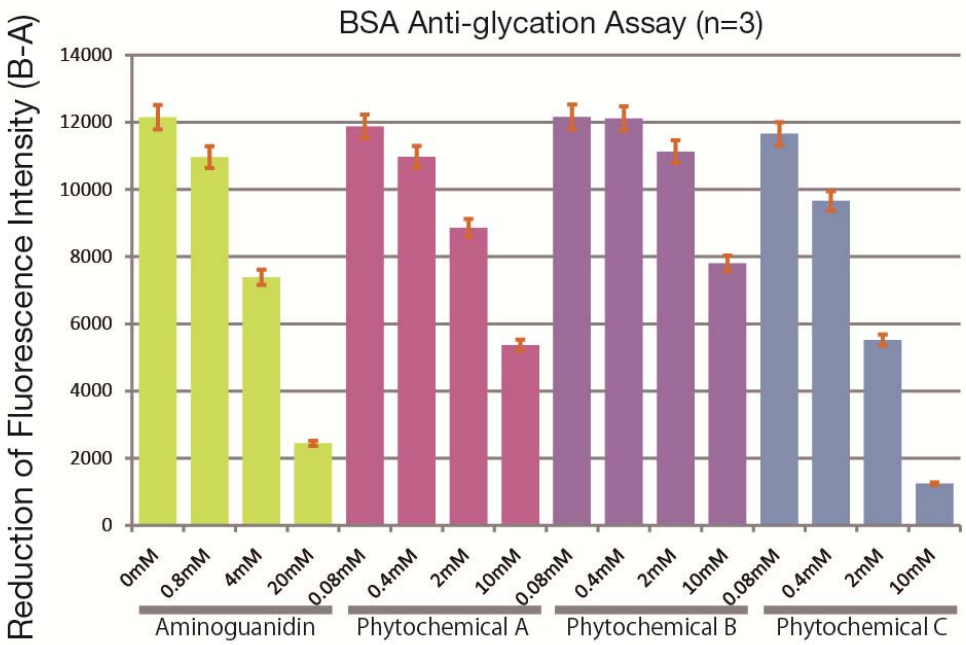
《Ⅱ. Assay protocol－96-well plate－》

(1) BSA solution is stored at room temperature (18 – 25 ℃) before testing.
(2) Add 50 uL of BSA solution to the 96-well black plate.
(3) Prepared Anti-glycation Standard (0.8 mM and 4mM) by diluting the 20 mM Aminoguanidine solution.
(4) Add 40 uL of the 0, 0.8, 4 and 20 mM Aminoguanidine (Anti-glycation Standard) or samples to each well. Dissolve the samples with the dilution buffer and filtrate with 0.22 um.
(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) Read the fluorescent intensity after 24 hrs 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 this kit results.



《Ⅳ．References》

(1) Masayoshi Takeuchi et al. Immunological Evidence that Non-carboxymethyllysine Advanced Glycation End-products Are Produced from Short Chain Sugars and Dicarbonyl Compounds in vivo. Mol Med. 2000 Feb;6(2):114-25. PMID: 10859028

(2) 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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COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
URL: http://www.cosmobio.co.jp e-mail: export@cosmobio.co.jp
[Outside Japan] Phone : +81-3-5632-9617 [国内連絡先] Phone : +81-3-5632-9610
FAX : +81-3-5632-9618 FAX : +81-3-5632-9619