COSMO BIO CO., LTD.

# Elastin Glycation Assay Kit, Glyceraldehyde

Cat. No. AAS-AGE-K05E

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## 【I - 1.】Background

Elastin is one of the extracellular matrix (ECM) proteins with the collagen that consists of many hydrophobic amino acids such as alanine, glycine, valine, and proline.
Elastin is the elastic fibrous protein, which located in aorta, ligaments, lung, skin, and connective tissue. It's wrapped around the coil-like collagen (Fig.1), to maintain the shape of the tissue and giving the elasticity-stretch.
Elastin is abundant in medial portion of the vessel wall, the content as arteries take is thick and blood pressure will be higher. Elasticity and flexibility acting on blood vessels are maintained by elastin (Fig. 2).

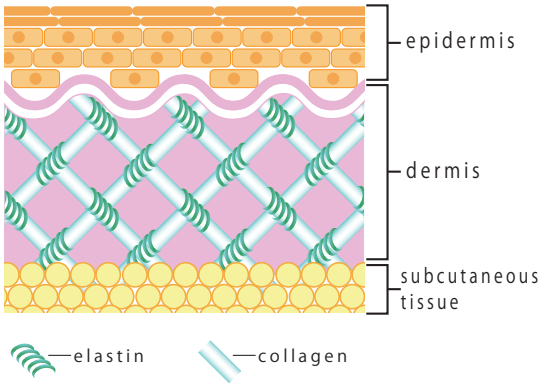



Fig.1 Elastin in skin tissue

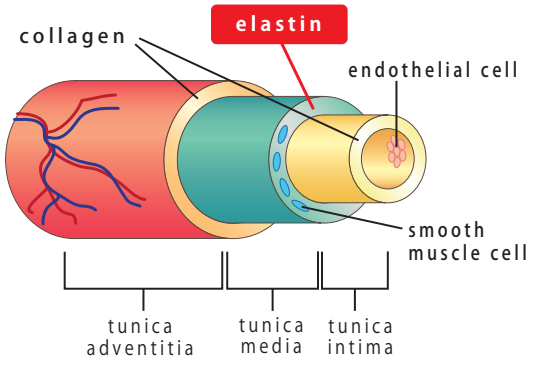



Fig. 2 Elastin in an artery wall

Elastin in the vessel wall and skin tissue is also glycated by diseases such as aging and diabetes. It has been reported to cause sclerotic change and aging of blood vessels and skin.
Elastin Glycation Assay Kit provides rapid detection of fluorescent AGEs found in elastin glycated with glyceraldehyde and screening of inhibitory effects of your samples. This kit provides sufficient reagents to perform up to 192 assays.
This kit is ideal for functional materials development that is focused such as lifestyle-related diseases and aging prevention research for blood vessels and ligaments.

## 【I - 2.】Assay Principle

Elastin Glycation Assay Kit is a complete assay system designed to measure the fluorescent AGEs formed in elastin, when the elastin is glycated with glyceraldehyde. The fluorescent AGEs can be detected with the fluorescence microplate reader equipped with a 370nm excitation filter and 440nm emission filter.

## 【 I - 3. 】 Kit Components

| No. | Component | Volume | Storage |
|---|---|---|---|
| 1 | Elastin Solution | 10 mL | 4℃ |
| 2 | Glyceraldehyde Solution（500mM） | 2 mL | |
| 3 | Dilution Buffer | 30 mL | |
| 4 | Aminoguanidine Solution（20mM）: Glycation Standard | 5 mL | |

* One kit contains regents for 192 assays (Tube or 96 well Plate)
* Additional materials required

## Required but not provided

・96well black plate（clear bottom、sterile）
Greiner [μ CLEAR — PLATE BLACK Cat.No.655090] is recommended.
・Fluorescent microplate reader
(Mode: Fluorescence Bottom Reading, Excitation Wavelength: 370nm, Emission Wavelength: 440nm）

## 【 II. 】 Assay Protocol

1. Elastin solution is stored at room temperature (18 – 25 ℃ ) before testing.
2. Add 50 uL of Elastin 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 3-6 days 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 3-6 days with a fluorescent microplate reader at 37℃ .
   Peg the fluorescence intensity at after incubation for 3-6 days 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.

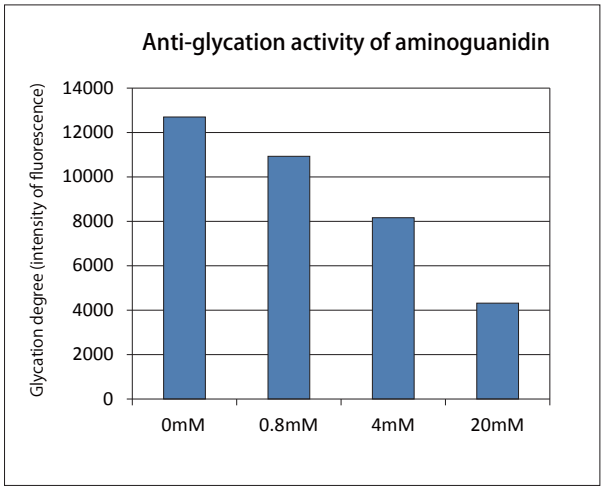
** In case samples contain florescent material, subtract the florescence intensity of the sample group without addition of glyceraldehyde (as "sample blank") from the group with glyceraldehyde.

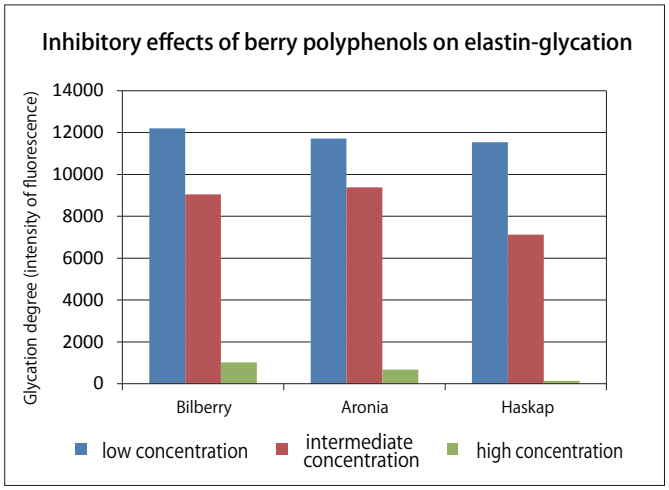


[Outside Japan] e-mail: export@cosmobio.co.jp Phone: +81-3-5632-9617 Fax: +81-3-5632-9618

## 【III.】Example of Results

The following figures demonstrate Elastin Glycation Assay Kit results.




## 【IV.】References

[1] E. Konova et al. Age-related changes in the glycation of human aortic elastin. Experimental Gerontology 39 (2004) 249-254

[2] G. Nicoloff et al. Serum AGE-elastin derived peptides among diabetic children. Vascular Pharmacology 43 (2005) 193-197



[Outside Japan] e-mail: export@cosmobio.co.jp Phone: +81-3-5632-9617 Fax: +81-3-5632-9618