COSMO BIO CO., LTD.

RAGE Reactive AGEs Assay Kit, Glyceraldehyde

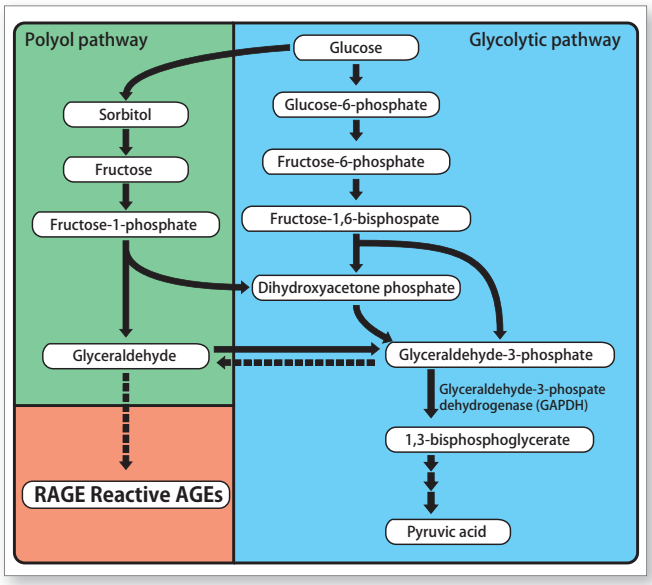
Cat. No. AAS-AGE-K04E

Edition Date : 2015 / 03 / 02

【 I. 】 Background

Although carbohydrates are indispensable for ATP production, excess carbohydrates promote protein glycation. Protein glycation may result in irreversible changes to protein structure, net charge, and protein function with deleterious consequences. Protein glycation has been associated with diabetic complications, renal disorders, Alzheimer's disease and others.
First described Louis Camille Maillard in 1912, glycation is also referred to as the Maillard reaction. Maillard reactions are categorized into early and advanced stages. Early stage reactions generate Amadori rearrangement products such as haemoglobin A1c, whereas advanced stage reactions generate the so-called AGEs (advanced glycation end products) characterized by protein-crosslinking and color browning. Collagens, important structural proteins of skin, blood vessel walls and bone, are subject to glycation.

Indeed, different types of receptors recognize AGEs as ligands. CD36, a scavenger receptor expressed on macrophages and vascular endothelial cells binds AGEs (mainly the CML form) and ox-LDL for clearing metabolic products resulting from oxidative stress. SR-A is also recognizes AGEs, Ox-LDL and Ac-LDL and this binding suggested a strong relationship between AGEs and arteriosclerotic diseases and diabetic nephropathy.
The so-called RAGE receptor (Receptor for AGEs) was found and isolated in 1992 from bovine lung as 55kDa membrane-spanning protein. (1) RAGE expression is distributed. RAGE is present not only on monocytes and or macrophages but also on vascular smooth muscle cells, neurons, hepatocytes, and kidney mesangial cells.



The function of AGE receptors are still unclear but have been suggested to effect signal transductions or the apoptosis process. RAGE itself does not bind binds to AGE types and exhibits high specificity to glycelaldehyde-derived AGEs (GA-AGE). GA-AGE has been suggested to be a "toxic AGE" (TAGE). Such TAGEs may be particularly detrimental to cells and may be more highly associated with disease or disease complications. In addition, recent reports describe glycosaminoglycan (GAG) sulfates on some cancer cells as RAGE ligands and suggest that such interactions may promote tumor metastasis (2,3).
Thus, factors effecting GA-AGE formation, and compounds effecting the interaction of GA-AGE with RAGE are of interest to study links between AGEs and disease and for the development of drugs that inhibit AGEs-related pathophysiologies.

(1) Schmidt, A. M. et al..,J. Biol. Chem. 267, 14987-14997, 1992
(2) S. Mizumoto et.al. J. Biol. Chem. 287(23) 18985-18994, 2012
(3) S. Mizumoto et.al. FEBS Journal., Vol. 280 Issue 10, p2462-2470, 2013

【I. -1】Kit Components

No.	Component	Volume	Storage
1	96-well albumin coated plate	1 Plate (One strip-well plate)	4℃
2	Microplate sealing film	96 well, 2 sheets	
3	Sample Dilution Buffer	30 mL	
4	Glyceraldehyde solution（100mM）	5 mL	
5	Aminoguanidine Stock Solution (20mM) ※ Positive control	0.5 mL	
6	Washing Buffer（10X）	30 mL	
7	Blocking Buffer	10 mL	
8	RAGE-Fc Solution	5 mL	
9	Alkaline Phosphatase (ALP) labeled protein A/G	5 mL	
10	Substrate Tablets (For 5mL)	3 tablets	
11	Substrate Dilution Buffer	15 mL	

【I. -2】Features

This kit is designed to detect GA-AGE formed on BSA using recombinant Fc-RAGE as detection reagent.
This kit will be useful not only for biomarker assessment and drug screening for drug development but also assessing the activity of functional foods and other ingested substances.

【II.】Assay Protocol

Materials Not Supplied

- Purified water
- 10μL ～ 1000μL micropipette
- 50μL ～ 200μL multi-channel micropipette
- Solution Reservoir
- Microplate reader（wavelength 405 nm）
- *37 ℃ humidified incubator
- 0.2μm filters

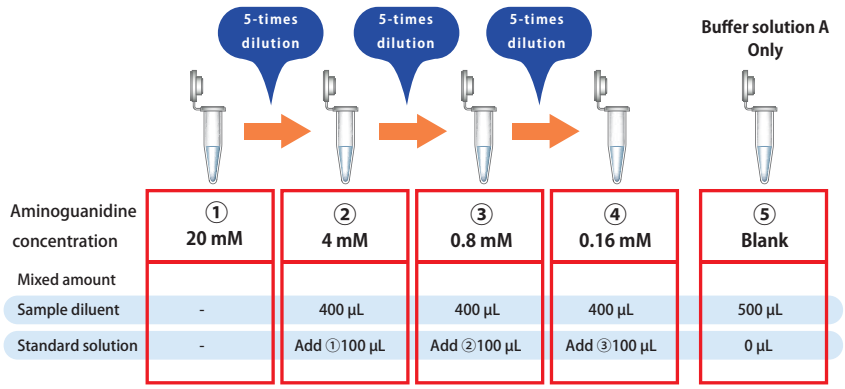
* Do not use CO_2 incubator. It affects solution pH and causes slow glycation.

Preparation of Reagents

- Aminoguanidine (positive control)

Stock Solutions

Dilute the 20 mM Aminoguanidine Stock Solution to 0 - 4mM with Sample Dilution Buffer as shown.



- Sample Solutions

Dilute each sample solution with Sample Dilution Buffer and filter through a 0.2μm filter.

- 1X Washing Buffer

Dilute the 10X Washing Buffer to 1X with purified water as needed.

- Substrate solution

Dissolve 1 Substrate Tablet with 5ml of Substrate Dilution Buffer.

Note: Substrate solution should be prepared just prior to use (see step 12).

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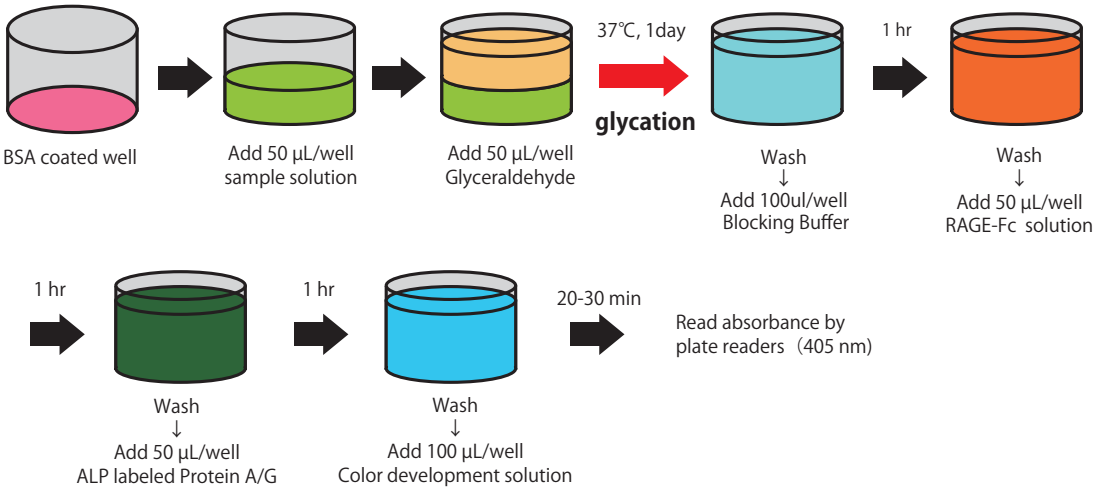
Protocol



1	Remove a suitable number of assay well strips from the aluminium pouch. Cut a piece of sealing film to the corresponding size.
2	Add 50 µl per well of the 0, 0.16, 0.8, 4 and 20mM Aminoguanidine Solution (positive control) or sample solution to each strip well.
3	Add 50 µl per well of 100mM Glyceraldehyde Solution.
4	Seal the plate well with the microplate seal and incubate under humid conditions for 24 hrs at 37℃ . Avoid evapolation. Do not use CO_2 incubator.
5	Remove the solution from each well completely and wash wells 3 times with 200ul/well of Washing Buffer. After the third wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Washing Buffer.
6	Add 100 µl/well of Blocking Buffer and incubate for 1hour at room temperature.
7	Remove the Blocking Buffer from each well completely and wash 3 times with 200 µl/well of Washing Buffer.
8	Add 50 µl/well of the RAGE-Fc Solution and incubate for 1hour at room temperature.
9	Remove the RAGE-Fc Solution from each well completely and wash 3 times with 200 µl/well of Washing Buffer.
10	Add 50 µl/well of the ALP labeled protein A/G and incubate for 1hour at room temperature.
11	Remove the ALP labeled protein A/G from each well completely and wash 3 times with 200 µl/well of Washing Buffer.
12	Add 100 µl/well of the Substrate solution and incubate at room temperature for 30 minutes. Raad absorbance of each well at 405nm.

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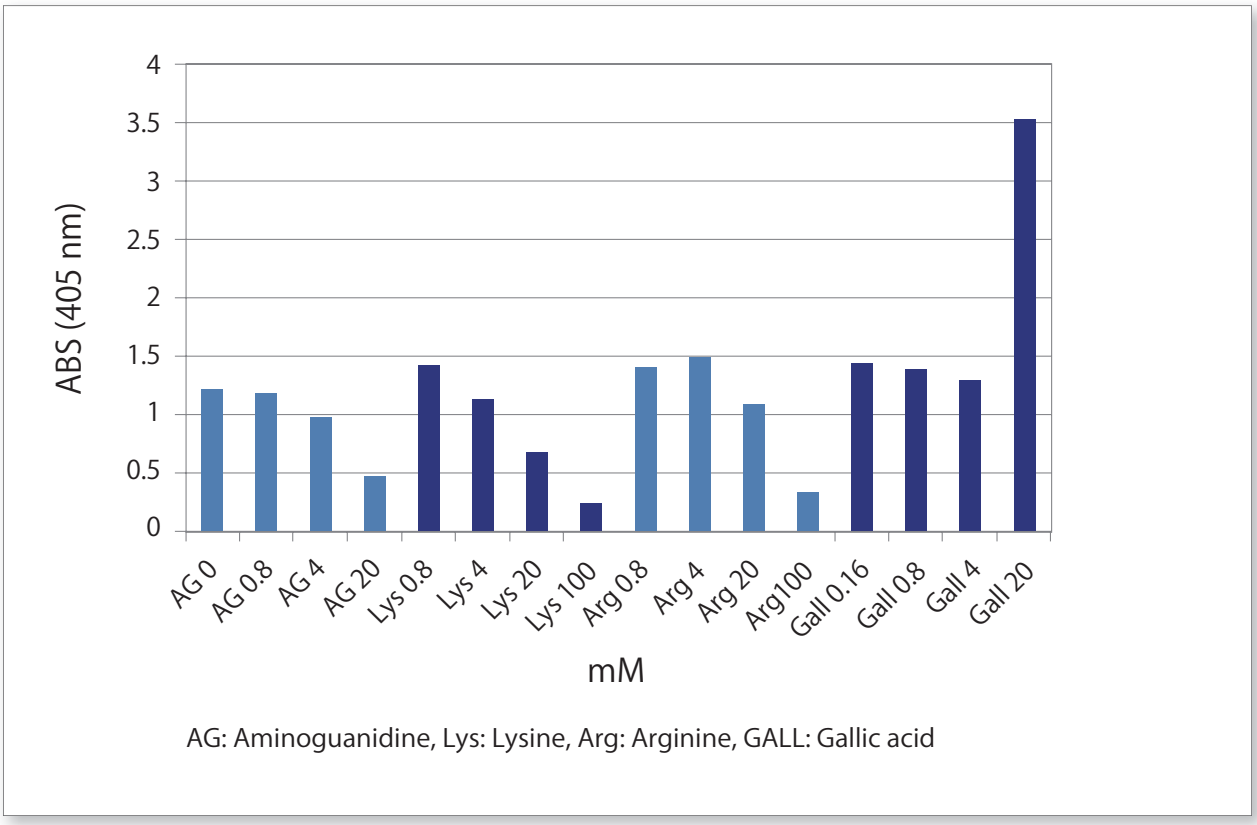
【 III. 】 Example of Results

Inhibition of RAGE reactive AGEs (GA-AGEs) formation by Aminoguanidine.
Lysine and arginine inhibit RAGE reactive AGEs formation with concentration dependent manner.



AG: Aminoguanidine, Lys: Lysine, Arg: Arginine, GALL: Gallic acid

【 IV. 】 References

[1] Schmidt, A. M. *et al., J. Biol. Chem.* **267**, 14987-14997, 1992.

[2] S. Mizumoto *et al. J. Biol. Chem.* **287** (23), 18985-18994, 2012.

[3] A. Nishikawa, T. Taira, K. Yoshizato. (1987) *In Vitro* Maturation of Collagen Fibrils Modulates Spreading, DNA Synthesis, and Collagenolysis of Epidermal Cells and Fibroblasts. *Exp. Cell Res.* **171**, p164-177.

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

[5] Jun-ichi Takino *et al.* (2010) The formation of intracellular glyceraldehyde-derived advanced glycation end-products and cytotoxicity. *J. Gastroenterol* **45**: 646-655.

[6] M. Takeuchi (2012) Participation of toxic AGEs (TAGE) in a variety of diseases. *Folia Pharmacol. Jpn.* **139**, p193-197.

[7] S. Mizumoto *et al. FEBS Journal.,* Vol. **280** Issue 10, p2462-2470, 2013.

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