Product Manual

OxiSelect™ Methylglyoxal (MG) Competitive ELISA Kit

Catalog Number

STA-811	96 assays
STA-811-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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, lipids and DNA. Tissue levels of AGE increase with age and the formation of AGEs is predominantly endogenous, though these products can also be derived from exogenous sources such as food and tobacco smoke. AGE modification of proteins can contribute to the pathophysiology of aging and long-term complications of diabetes, atherosclerosis and renal failure. AGEs also interact with a variety of cell-surface AGE-binding receptors (RAGE), leading either to their endocytosis and degradation or to cellular activation and pro-oxidant or pro-inflammatory events.

Several AGE structures have been reported, such as N^{ε}-(carboxymethyl) lysine (CML), N^{ε}-(carboxyethyl) lysine (CEL), pentosidine, and Methylglyoxal (MG) derivatives. MG is formed through non-oxidative mechanisms from triose phosphates during anaerobic glycolysis and it can modify amino acids, nucleic acids, and proteins. MG reacts with arginine, lysine and cysteine residues of proteins to form AGEs. MG is involved in various pathological processes. For example, MG derivatives are found elevated in diabetes.

The OxiSelect™ Methylglyoxal (MG) ELISA Kit is an enzyme immunoassay developed for rapid detection and quantitation of MG-H1 (methyl-glyoxal-hydro-imidazolone) protein adducts. The quantity of MG adduct in protein samples is determined by comparing its absorbance with that of a known MG-BSA standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown protein samples.

Assay Principle

First, an MG conjugate is coated on the ELISA plate. The unknown MG protein samples or MG-BSA standards are then added to the MG conjugate preabsorbed plate. After a brief incubation, the anti-MG antibody is added, followed by an HRP conjugated secondary antibody. The content of MG protein adducts in unknown samples is determined by comparison with the predetermined MG-BSA standard curve.

Related Products

1. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
2. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
3. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG)
4. STA-816: OxiSelect™ N^{ε}-(carboxymethyl) lysine (CML) Competitive ELISA Kit
5. STA-817: OxiSelect™ Advanced Glycation End Products (AGE) Competitive ELISA Kit
6. STA-832: OxiSelect™ MDA Adduct Competitive ELISA Kit
7. STA-838: OxiSelect™ HNE Adduct Competitive ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate
2. Anti-MG Antibody (1000X) (Part No. 281101): One 10 µL vial of anti-MG antibody
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): One 20 µL vial
4. Assay Diluent (Part No. 310804): One 50 mL bottle
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle
7. Stop Solution (Part. No. 310808): One 12 mL bottle

Box 2 (shipped on blue ice packs)

1. MG-BSA Standard (Part No. 281102): One 75 µL vial of 1.0 mg/mL MG-BSA in PBS
2. MG Conjugate (Part No. 281103): One 20 µL vial of MG conjugate at 1.0 mg/mL in PBS
3. 100X Conjugate Diluent (Part No. 281603): One 300 µL vial

Materials Not Supplied

1. Protein samples such as purified protein, plasma, serum, cell lysate
2. 1X PBS
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Anti-MG Antibody, MG-BSA Standard, MG Conjugate and 100X Conjugate Diluent at -20ºC to avoid multiple freeze/thaw cycles. Store all other kit components at 4ºC.

Preparation of Reagents

- MG Conjugate Coated Plate:

  Note: The MG Conjugate coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.

  1. Immediately before use, prepare 1X Conjugate Diluent by diluting the 100X Conjugate Diluent in 1X PBS. Example: Add 50 µL to 4.95 mL of 1X PBS.

2. Immediately before use, prepare 500 ng/mL of MG Conjugate by diluting the 1.0 mg/mL MG Conjugate in 1X Conjugate Diluent in two step dilutions. Example: Add 5 µL of 1.0 mg/mL MG Conjugate to 995 µL of 1X PBS, vortex thoroughly, and transfer 500 µL to another tube containing 4.5 mL of 1X Conjugate Diluent.
3. Add 100 µL of the **500 ng/mL** MG Conjugate to each well to be tested and incubate overnight at 4ºC. Remove the MG Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hr at room temperature on an orbital shaker. Transfer the plate to 4ºC and remove the Assay Diluent **immediately before use.**

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-MG Antibody and Secondary Antibody: Immediately before use, dilute the Anti-MG antibody 1:1000 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of MG-BSA standards in the concentration range of 0 to 25 µg/mL by diluting the 1 mg/mL MG-BSA standard in Assay Diluent. Example: Add 10 µL to 390 µL of Assay Diluent. Further prepare a series of MG-BSA standards according to Table 1.

Standard Tubes	1 mg/mL MG-BSA Standard (µL)	Assay Diluent (µL)	MG-BSA (µg/mL)
1	10	390	25
2	200 of tube #1	200	12.5
3	200 of tube #2	200	6.25
4	200 of tube #3	200	3.13
5	200 of tube #4	200	1.56
6	200 of tube #5	200	0.78
7	200 of tube #6	200	0.39
8	200 of tube #7	200	0.20
9	0	200	0

Table 1. Preparation of MG-BSA Standard Curve

Assay Protocol

Note: If testing mouse or rat plasma or serum, the IgG must be completely removed from each sample prior to testing, such as with Protein A or G beads. Additionally, a control well without primary antibody should be run for each sample to determine background signal.

1. Prepare and mix all reagents thoroughly before use. Each MG sample including unknown and standard should be assayed in duplicate.

2. Add 50 µL of unknown sample or MG-BSA standard to the wells of the MG Conjugate coated plate. If needed, unknown samples may be diluted in 1X PBS containing 0.1% BSA before adding. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 µL of the diluted anti-MG antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash 3 times with 250 µL of 1X Wash Buffer with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Secondary Antibody-HRP Conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 5 times according to step 4 above.
6. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well. Incubate at room temperature for 2-20 minutes on an orbital shaker.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
7. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).
8. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Methylglyoxal (MG) Competitive ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

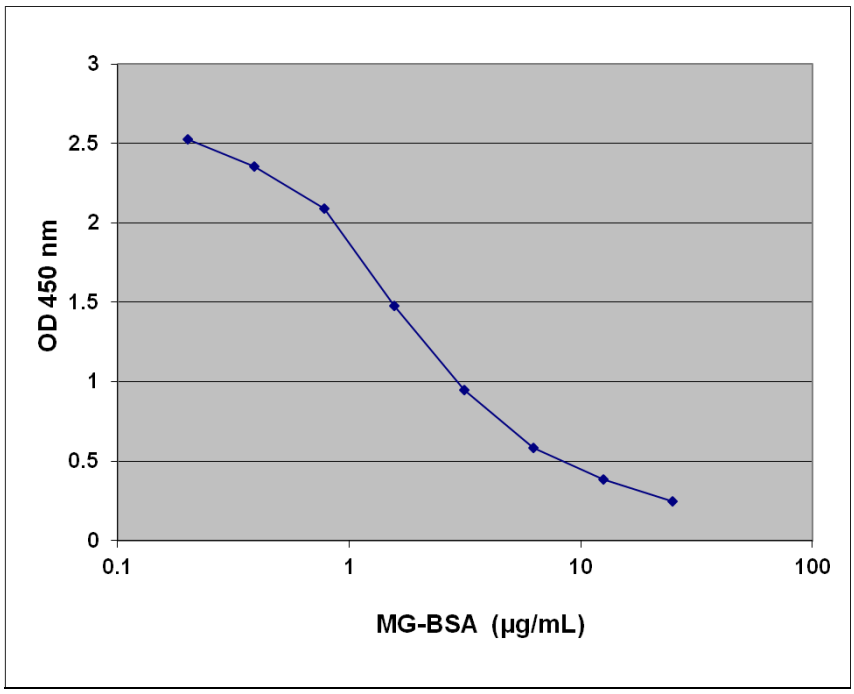

Figure 1: MG-BSA Competitive ELISA Standard Curve.

Cross reactivity of Methylglyoxal (MG) Competitive ELISA Kit

AGEs	Cross Reactivity
MG-BSA	100%
AGE-BSA*	2.3%
CML-BSA	<0.001%
CEL-BSA	<0.001%
BSA	<0.001%
Ovalbumin	<0.001%

* AGE-BSA is prepared by incubating BSA with D-Glucose at 37ºC for 6 weeks under sterile conditions.

References

1. Shamshi F. A, Partal A, Sady C, Glomb M. A, Nagaraj R. H (1998) *J Biol Chem* **273**:6928-6936.
2. Cai W, Gao Q. D, Zhu L, Peppa M, He C, Vlassara H. (2002), *Mol Med.* **8**:337-46.
3. Monnier, V., and Cerami, A. (1981) *Science* **211,** 491–493.
4. Ahmed M.U., Thorpe S.R., Baynes J.W (1986) *J. Biol. Chem.* **261**, 4889–4894.
5. Reddy S., Bichler J., Wells-Knecht K.J., Thorpe S.R., Baynes J.W (1995) *Biochemistry* **34**, 10872–10878.
6. Dunn, J. A., Patrick, J. S., Thorpe, S. R., and Baynes, J. W. (1989) *Biochemistry* **28**, 9464-9468.

7. Ahmed, M. U., Brinkmann Frye, E., Degenhardt, T. P., Thorpe, S. R., and Baynes, J. W. (1997) *Biochem. J.* **324**, 565-570.
8. Sell, D. R., and Monnier, V. M. (1989) *J. Biol. Chem.* **264**, 21597-21602.
9. Onorato, J., Jenkins, A., Thorpe, S., and Baynes, J. (2000) *J. Biol. Chem.* **275,** 21177–21184.
10. Boehm BO, Schilling S, Rosinger S, Lang GE, Lang GK, Kientsch-Engel P, Stahl P (2004) *Diabetologia* **47**, 1376–1379.

Recent Product Citations

1. Gutierrez-Mariscal, F.M. et al. (2020). Reduction in Circulating Advanced Glycation End Products by Mediterranean Diet is Associated with Increased Likelihood of type 2 Diabetes Remission in Patients with Coronary Heart Disease: From the Cordioprev Study. *Mol Nutr Food Res*. doi: 10.1002/mnfr.201901290.
2. Li, J. et al. (2020). Renal protective effects of empagliflozin via inhibition of EMT and aberrant glycolysis in proximal tubules. *JCI Insight*. pii: 129034. doi: 10.1172/jci.insight.129034.
3. Piuri, G. et al. (2020). Methylglyoxal, Glycated Albumin, PAF, and TNF-α: Possible Inflammatory and Metabolic Biomarkers for Management of Gestational Diabetes. *Nutrients*. **12**:479. doi: 10.3390/nu12020479.
4. Shimizu, Y. et al. (2020). Role of DJ-1 in Modulating Glycative Stress in Heart Failure. *J Am Heart Assoc*. **9**(4). doi: 10.1161/jaha.119.014691.
5. de la Cruz-Ares, S. et al. (2020). Endothelial Dysfunction and Advanced Glycation End Products in Patients with Newly Diagnosed Versus Established Diabetes: From the CORDIOPREV Study. *Nutrients*. **12**(1). pii: E238. doi: 10.3390/nu12010238.
6. Liu, C. et al. (2020). Inhibition of thioredoxin 2 by intracellular methylglyoxal accumulation leads to mitochondrial dysfunction and apoptosis in INS-1 cells. *Endocrine*. doi: 10.1007/s12020-020-02191-x.
7. Egawa, T. et al. (2019). The Protective Effect of Brazilian Propolis against Glycation Stress in Mouse Skeletal Muscle. *Foods*. **8**(10). pii: E439. doi: 10.3390/foods8100439.
8. Do, M.H. et al. (2019). Schizonepeta tenuifolia reduces methylglyoxal-induced cytotoxicity and oxidative stress in mesangial cells. *J Funct Foods*. doi: 10.1016/j.jff.2019.103531.
9. Nakamura, T. et al. (2019). Poorly controlled type 2 diabetes with no progression of diabetes-related complications and low levels of advanced glycation end products: A Case report. *Medicine (Baltimore)*. **98**(30):e16573. doi: 10.1097/MD.0000000000016573.
10. Griggs, R.B. et al. (2019). Methylglyoxal and a spinal TRPA1-AC1-Epac cascade facilitate pain in the db/db mouse model of type 2 diabetes. *Neurobiol Dis*. **127**:76-86. doi: 10.1016/j.nbd.2019.02.019.
11. Shamsaldeen, Y.A. et al. (2019). Dysfunction in nitric oxide synthesis in streptozotocin treated rat aorta and role of methylglyoxal. *Eur J Pharmacol*. **842**:321-328. doi: 10.1016/j.ejphar.2018.10.056.
12. Simón, L. et al. (2018). Olive oil addition to the high-fat diet reduces methylglyoxal (MG-H1) levels increased in hypercholesterolemic rabbits. *Mediterranean Journal of Nutrition and Metabolism*. 1-9. doi:10.3233/mnm-180229.
13. Thompson, K. et al. (2018). Advanced glycation end (AGE) product modification of laminin downregulates Kir4.1 in retinal Müller cells. *PLoS One*. **13**(2):e0193280. doi: 10.1371/journal.pone.0193280.

14. Suh, K.S. et al. (2018). Cytoprotective effects of xanthohumol against methylglyoxal-induced cytotoxicity in MC3T3-E1 osteoblastic cells. *J Appl Toxicol*. **38**:180–192.
15. Park, S. et al. (2017). Bariatric Surgery can Reduce Albuminuria in Patients with Severe Obesity and Normal Kidney Function by Reducing Systemic Inflammation. *Obes Surg*. doi: 10.1007/s11695-017-2940-y.
16. Suh, K.S. et al. (2017). Magnolol protects pancreatic β-cells against methylglyoxal-induced cellular dysfunction. *Chem Biol Interact*. **277**:101-109. doi: 10.1016/j.cbi.2017.09.014.
17. Suh, K.S. et al. (2017). Limonene protects osteoblasts against methylglyoxal-derived adduct formation by regulating glyoxalase, oxidative stress, and mitochondrial function. *Chem Biol Interact*. **278**:15-21. doi: 10.1016/j.cbi.2017.10.001.
18. Suh, K.S. et al. (2017). Deoxyactein protects pancreatic β-cells against methylglyoxal-induced oxidative cell damage by the upregulation of mitochondrial biogenesis. *Int. J. Mol. Med.* doi:10.3892/ijmm.2017.3018.
19. Nishimoto, S. et al. (2017). Activation of Nrf2 attenuates carbonyl stress induced by methylglyoxal in human neuroblastoma cells: Increase in GSH levels is a critical event for the detoxification mechanism. *Biochem Biophys Res Commun*. **483**(2):874-879. doi: 10.1016/j.bbrc.2.
20. Lopez-Moreno, J. et al. (2016). Mediterranean diet supplemented with Coenzyme Q10 modulates the postprandial metabolism of advanced glycation end products in elderly men and women. *J. Gerontol. A Biol. Sci. Med. Sci*. doi:10.1093/gerona/glw214.
21. Ueda, K. et al. (2016). Photodegradation of retinal bisretinoids in mouse models and implications for macular degeneration. *Proc Natl Acad Sci U S A.* doi: 10.1073/pnas.1524774113.
22. Morgan, P. E. et al. (2014). Perturbation of human coronary artery endothelial cell redox state and NADPH generation by methylglyoxal. *PLoS One*. **9**:e86564.

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