Product Manual

OxiSelect™ Advanced Glycation End Product (AGE) Competitive ELISA Kit

Catalog Number

STA-817	96 assays
STA-817-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

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. For example, *N*-ε-(Carboxymethyl) lysine (CML), one of the prevalent AGEs, has been implicated in oxidative stress and vascular damage. 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.

Cell Biolabs' OxiSelect™ AGE Competitive ELISA Kit is an enzyme immunoassay developed for rapid detection and quantitation of AGE protein adducts. The quantity of AGE adduct in protein samples is determined by comparing its absorbance with that of a known AGE-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 AGE conjugate is coated on an ELISA plate. The unknown AGE protein samples or AGE-BSA standards are then added to the AGE conjugate preabsorbed ELISA plate. After a brief incubation, an anti-AGE polyclonal antibody is added, followed by an HRP conjugated secondary antibody. The content of AGE protein adducts in unknown samples is determined by comparison with a predetermined AGE-BSA standard curve.

Related Products

1. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
2. STA-811: OxiSelect™ Methylglyoxal (MG) Competitive ELISA Kit
3. STA-813: OxiSelect™ N^{ε}-(carboxyethyl) lysine (CEL) Competitive ELISA Kit
4. STA-816: OxiSelect™ N^{ε}-(carboxymethyl) lysine (CML) Competitive ELISA Kit
5. STA-832: OxiSelect™ MDA 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-AGE Antibody (1000X) (Part No. 281701): One 10 µL vial of anti-AGE antibody.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 231704): 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. AGE-BSA Standard (Part No. 281703): One 125 µL vial of 1 mg/mL AGE-BSA in PBS.
2. AGE Conjugate (Part No. 281702): One 50 µL vial of AGE 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-AGE Antibody, AGE-BSA Standard, AGE Conjugate and 100X Conjugate Diluent at -20ºC to avoid multiple freeze/thaw cycles. Store all other kit components at 4ºC.

Preparation of Reagents

- AGE Conjugate Coated Plate:

  Note: The AGE 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 10 µg/mL of AGE Conjugate by diluting the 1.0 mg/mL AGE Conjugate in 1X PBS. Example: Add 25 µL to 2.475 mL of 1X PBS.

3. Mix 10 µg/mL of AGE Conjugate and 1X Conjugate Diluent at 1:1 ratio and add 100 µL of the mixture to each well and incubate overnight at 4ºC. Remove the AGE 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. Transfer the plate to 4ºC and remove the Assay Diluent **immediately before use**.

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Anti-AGE Antibody and Secondary Antibody: Immediately before use, dilute the Anti-AGE 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 AGE-BSA standards in the concentration range of 0 to 100 µg/mL by diluting the AGE-BSA Standard in Assay Diluent (Table 1).

Standard Tubes	1 mg/mL AGE-BSA Standard (µL)	Assay Diluent (µL)	AGE-BSA (µg/mL)
1	40	360	100
2	200 of Tube #1	200	50
3	200 of Tube #2	200	25
4	200 of Tube #3	200	12.5
5	200 of Tube #4	200	6.25
6	200 of Tube #5	200	3.13
7	200 of Tube #6	200	1.56
8	200 of Tube #7	200	0.78
9	200 of Tube #8	200	0.39
10	0	200	0

Table 1. Preparation of AGE-BSA Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each AGE sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or AGE-BSA standard to the wells of the AGE 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-AGE 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 3 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 2-30 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 AGE Competitive ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

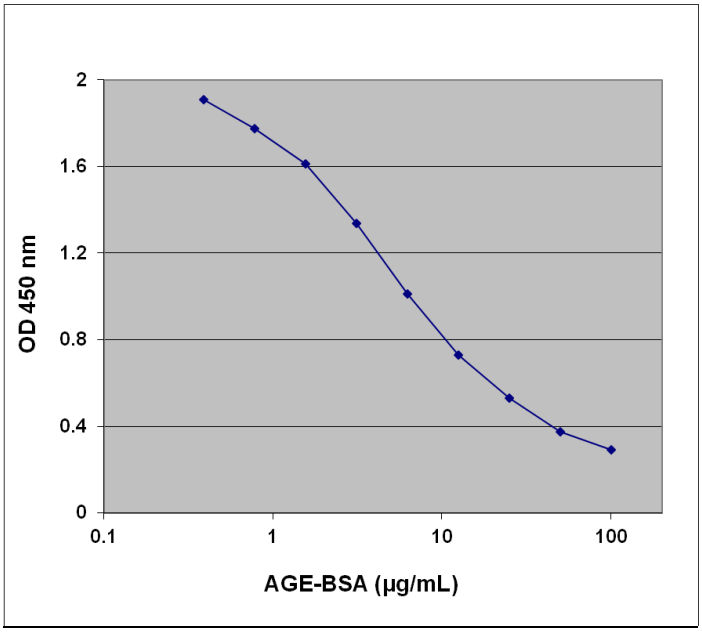

Figure 1: AGE-BSA Competitive ELISA Standard Curve.

References

1. Monnier, V., and Cerami, A. (1981) *Science* **211,** 491–493.
2. Dunn, J. A., Patrick, J. S., Thorpe, S. R., and Baynes, J. W. (1989) *Biochemistry* **28**, 9464-9468.

3. Ahmed, M. U., Brinkmann Frye, E., Degenhardt, T. P., Thorpe, S. R., and Baynes, J. W. (1997) *Biochem. J.* **324**, 565-570.
4. Sell, D. R., and Monnier, V. M. (1989) *J. Biol. Chem.* **264**, 21597-21602.
5. Onorato, J., Jenkins, A., Thorpe, S., and Baynes, J. (2000) *J. Biol. Chem.* **275,** 21177–21184.

Recent Product Citations

1. Hassan, M.A. et al. (2023). The Synergistic Influence of Polyflavonoids from Citrus aurantifolia on Diabetes Treatment and Their Modulation of the PI3K/AKT/FOXO1 Signaling Pathways: Molecular Docking Analyses and In Vivo Investigations. *Pharmaceutics*. **15**(9):2306. doi : 10.3390/pharmaceutics15092306.
2. Liu, SH. et al. (2023). Attenuation of diabetes-mediated muscle atrophy in rats by fish oil enriched omega-3 polyunsaturated fatty acids supplementation. *J Food Drug Anal*. **31**(3):6. doi: 10.38212/2224-6614.3468.
3. Yamamoto, T. et al. (2023). Protein components of maple syrup as a potential resource for the development of novel anti-colorectal cancer drugs. *Oncol Rep*. **50**(4):179. doi: 10.3892/or.2023.8616.
4. Dewidar, B. et al. (2023). Alterations of hepatic energy metabolism in murine models of obesity, diabetes and fatty liver diseases. *EBioMedicine*. **94**:104714. doi: 10.1016/j.ebiom.2023.104714.
5. Jorge-Galarza, E. et al. (2023). Adipose tissue dysfunction serum markers are associated with high density lipoprotein size and glycation in the early stages of type 2 diabetes. *Lipids Health Dis*. **22**(1):89. doi: 10.1186/s12944-023-01847-7.
6. Lan, K.C. et al. (2023). Resveratrol Alleviates Advanced Glycation End-Products-Related Renal Dysfunction in D-Galactose-Induced Aging Mice. *Metabolites*. **13**(5):655. doi: 10.3390/metabo13050655.
7. Tripathi, D. et al. (2023). Oxidative and Glycation Damage to Mitochondrial DNA and Plastid DNA during Plant Development. *Antioxidants (Basel)*. **12**(4):891. doi: 10.3390/antiox12040891.
8. Park, Y.S. et al. (2023). Pyruvate Kinase M2: A New Biomarker for the Early Detection of Diabetes-Induced Nephropathy. *Int J Mol Sci*. **24**(3):2683. doi: 10.3390/ijms24032683.
9. Pavlovic, D. et al. (2023). The GSTO2 (rs156697) Polymorphism Modifies Diabetic Nephropathy Risk. *Medicina (Kaunas)*. **59**(1):164. doi: 10.3390/medicina59010164.
10. Kundu, A. et al. (2022). Tenovin-1 Ameliorates Renal Fibrosis in High-Fat-Diet-Induced Diabetic Nephropathy via Antioxidant and Anti-Inflammatory Pathways. *Antioxidants (Basel)*. **11**(9):1812. doi: 10.3390/antiox11091812.
11. Wu, H.P. et al. (2022). Decreased Monocyte HLA-DR Expression in Patients with Sepsis and Acute Kidney Injury. *Medicina (Kaunas)*. **58**(9):1198. doi: 10.3390/medicina58091198.
12. Pramanik, S. et al. (2022). The Amelioration of Detrimental Biochemical Anomalies by Supplementing B, C, and E Vitamins in Subjects with Type 2 Diabetes Mellitus May Reduce the Rate of Development of Diabetic Retinopathy. *J Diabetes Res*. doi: 10.1155/2022/3886710.
13. Zych, M. et al. (2022). Two Bioactive Compounds, Rosmarinic Acid And Sinapic Acid, Do Not Affect The Depleted Glutathione Level In The Lenses Of Type 2 Diabetic Female Rats. *Farmacia*. **70**(4):607-616. doi: 10.31925/farmacia.2022.4.5.
14. El-Sayed, N.S. et al. (2022). Potential mechanisms underlying the association between type II diabetes mellitus and cognitive dysfunction in rats: a link between miRNA-21 and Resveratrol's neuroprotective action. *Metab Brain Dis*. doi: 10.1007/s11011-022-01035-z.
15. Lim, O. et al. (2022). Stingless bee propolis, metformin, and their combination alleviate diabetic cardiomyopathy. *Brazilian J. Pharm. Sci*. doi: 10.1590/s2175-97902022e19652.

16. Sabbatinelli, J. et al. (2022). Circulating levels of AGEs and soluble RAGE isoforms are associated with all-cause mortality and development of cardiovascular complications in type 2 diabetes: a retrospective cohort study. *Cardiovasc Diabetol*. **21**(1):95. doi: 10.1186/s12933-022-01535-3.
17. Tan, H. et al. (2022). Glabridin, a bioactive component of licorice, ameliorates diabetic nephropathy by regulating ferroptosis and the VEGF/Akt/ERK pathways. *Mol Med*. **28**(1):58. doi: 10.1186/s10020-022-00481-w.
18. Gu, M.J. et al. (2022). Glycolaldehyde, an Advanced Glycation End Products Precursor, Induces Apoptosis via ROS-Mediated Mitochondrial Dysfunction in Renal Mesangial Cells. *Antioxidants*. **11**(5):934. doi: 10.3390/antiox11050934.
19. Wu, T.H. et al. (2022). Increased serum levels of advanced glycation end products are negatively associated with relative muscle strength in patients with type 2 diabetes mellitus. *BMC Endocr Disord*. **22**(1):118. doi: 10.1186/s12902-022-01035-1.
20. Wu, H.P. et al. (2022). Increased Production of Interleukin-10 and Tumor Necrosis Factor-Alpha in Stimulated Peripheral Blood Mononuclear Cells after Inhibition of S100A12. *Curr Issues Mol Biol*. **44**(4):1701-1712. doi: 10.3390/cimb44040117.
21. Borymska, W. et al. (2022). Silymarin from Milk Thistle Fruits Counteracts Selected Pathological Changes in the Lenses of Type 1 Diabetic Rats. *Nutrients*. **14**(7):1450. doi: 10.3390/nu14071450.
22. Abdou, H.M. et al. (2022). Antidiabetic efficacy of Trifolium alexandrinum extracts hesperetin and quercetin in ameliorating carbohydrate metabolism and activating IR and AMPK signaling in the pancreatic tissues of diabetic rats. *Biomed Pharmacother*. **149**:112838. doi: 10.1016/j.biopha.2022.112838.
23. Karamalakova, Y. et al. (2022). Pulmonary Protein Oxidation and Oxidative Stress Modulation by Lemna minor L. in Progressive Bleomycin-Induced Idiopathic Pulmonary Fibrosis. *Antioxidants (Basel)*. **11**(3):523. doi: 10.3390/antiox11030523.
24. Tripathi, D. et al. (2022). Glycation damage to organelles and their DNA increases during maize seedling development. *Sci Rep*. **12**(1):2688. doi: 10.1038/s41598-022-06454-7.
25. Schadler, P. et al. (2022). The Association of Blood Biomarkers and Body Mass Index in Knee Osteoarthritis: A Cross-Sectional Study. *Cartilage*. **13**(1):19476035211069251. doi: 10.1177/19476035211069251.
26. Krisanits, B.A. et al. (2022). Non-enzymatic glycoxidation linked with nutrition enhances the tumorigenic capacity of prostate cancer epithelia through AGE mediated activation of RAGE in cancer associated fibroblasts. *Transl Oncol*. **17**:101350. doi: 10.1016/j.tranon.2022.101350.
27. Tsai, P.S. et al. (2021). Advanced glycation end products activated endothelial-to-mesenchymal transition in pancreatic islet endothelial cells and triggered islet fibrosis in diabetic mice. *Chem Biol Interact*. doi: 10.1016/j.cbi.2021.109562.
28. Igaki, Y. et al. (2021). Xanthine oxidoreductase-mediated injury is amplified by upregulated AMP deaminase in type 2 diabetic rat hearts under the condition of pressure overload. *J Mol Cell Cardiol*. doi: 10.1016/j.yjmcc.2021.01.002.
29. Pantner, Y. et al. (2021). DJ-1 attenuates the glycation of mitochondrial complex I and complex III in the post-ischemic heart. *Sci Rep*. **11**(1):19408. doi: 10.1038/s41598-021-98722-1.
30. Waditee-Sirisattha, R. & Kageyama, H. (2021). Protective effects of mycosporine-like amino acid-containing emulsions on UV-treated mouse ear tissue from the viewpoints of antioxidation and antiglycation. *J Photochem Photobiol B*. **223**:112296. doi: 10.1016/j.jphotobiol.2021.112296.

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