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Product Manual

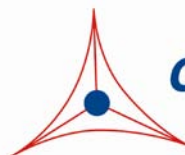
# OxiSelect™ Advanced Glycation End Product (AGE) Competitive ELISA Kit

## Catalog Number

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

**FOR RESEARCH USE ONLY**  
Not for use in diagnostic procedures

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**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*- $\epsilon$ -(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-300: OxiSelect™ N<sup>ε</sup>-(carboxyethyl) lysine (CEL) ELISA Kit
2. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
3. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
4. STA-311: OxiSelect™ Methylglyoxal (MG) ELISA Kit
5. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
6. STA-334: OxiSelect™ HNE-His Adduct ELISA Kit
7. STA-816: OxiSelect™ N<sup>ε</sup>-(carboxymethyl) lysine (CML) Competitive ELISA Kit
8. STA-832: OxiSelect™ MDA Adduct Competitive ELISA Kit
9. 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-AGE Antibody (1000X) (Part No. 281701): One 10  $\mu$ L vial of anti-AGE antibody.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 231704): One 20  $\mu$ L 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  $\mu$ L vial of 1 mg/mL AGE-BSA in PBS.
2. AGE Conjugate (Part No. 281702): One 50  $\mu$ L vial of AGE conjugate at 1.0 mg/mL in PBS.
3. 100X Conjugate Diluent (Part No. 281603): One 300  $\mu$ L vial.

## **Materials Not Supplied**

1. Protein samples such as purified protein, plasma, serum, cell lysate
2. 1X PBS
3. 10  $\mu$ L to 1000  $\mu$ L adjustable single channel micropipettes with disposable tips
4. 50  $\mu$ L to 300  $\mu$ L 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 -20°C to avoid multiple freeze/thaw cycles. Store all other kit components at 4°C.

## **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  $\mu$ L to 4.95 mL of 1X PBS.
2. Immediately before use, prepare 10  $\mu$ g/mL of AGE Conjugate by diluting the 1.0 mg/mL AGE Conjugate in 1X PBS. Example: Add 25  $\mu$ L 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 4°C. 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 4°C 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).

<b>Standard Tubes</b>	<b>1 mg/mL AGE-BSA Standard (µL)</b>	<b>Assay Diluent (µL)</b>	<b>AGE-BSA (µg/mL)</b>
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**

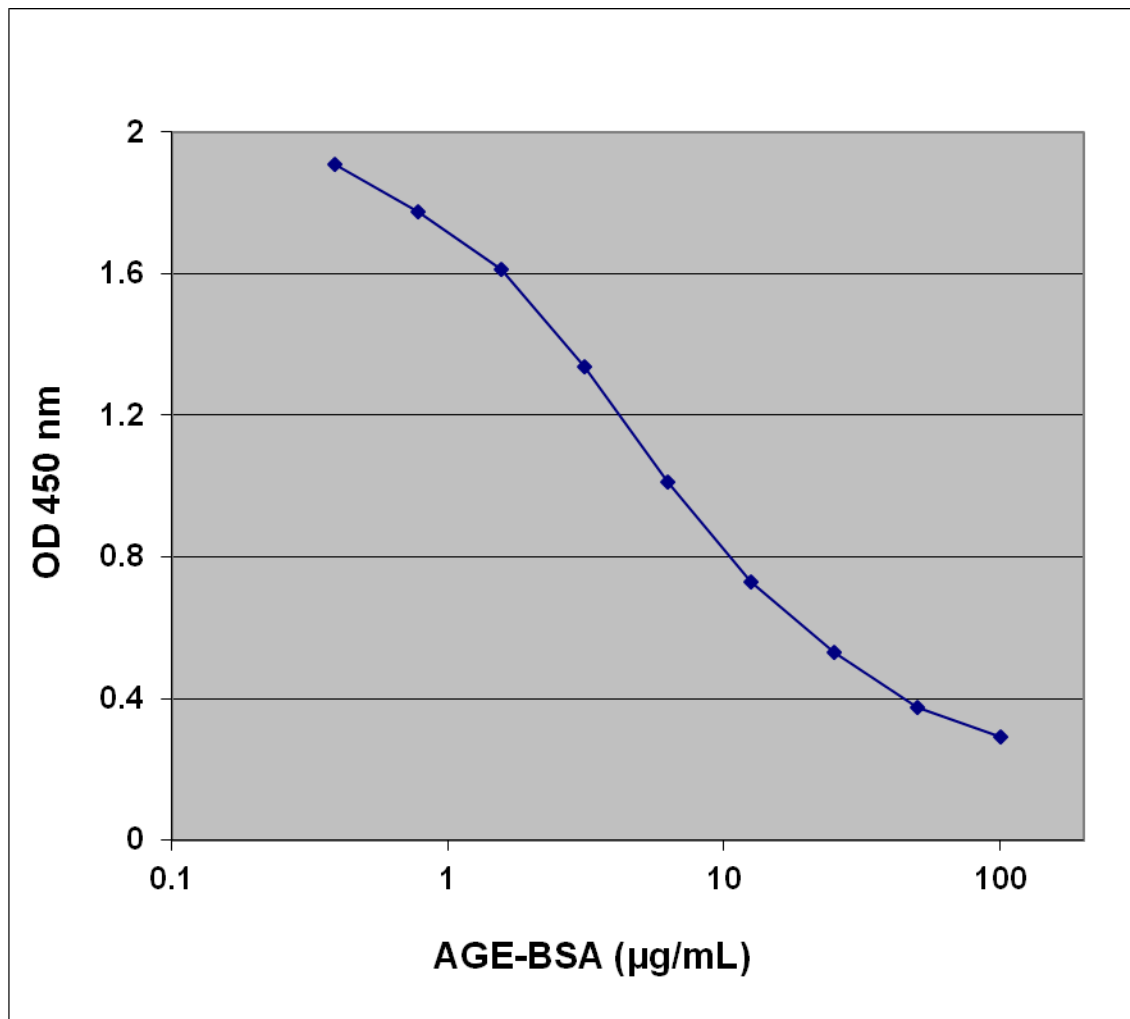
### **Assay Protocol**

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  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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.

## **Warranty**

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