Product Manual

OxiSelect™ Advanced Glycation End Product (AGE) ELISA Kit

Catalog Number

STA-317 **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. 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' AGE 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

AGE-BSA standards or protein samples (10 µg/mL) are adsorbed onto a 96-well plate for 2 hrs at 37ºC. The AGE protein adducts present in the sample or standard are probed with an anti-AGE polyclonal antibody, followed by an HRP conjugated secondary antibody. The AGE protein adduct content in an unknown sample is determined by comparing with a standard curve that is prepared from AGE-BSA standards. AGE-BSA was prepared by reacting BSA with glycolaldehyde, and followed by extensive dialysis and column purification. It contains CML, pentosidine and other AGE structures. The same AGE-BSA was used as antigen to prepare the polyclonal antibody used in the AGE ELISA Kit.

Related Products

1. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
2. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
3. STA-318: OxiSelect™ AOPP Assay Kit
4. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG)
5. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
6. STA-332: OxiSelect™ MDA Adduct ELISA Kit
7. STA-334: OxiSelect™ HNE-His Adduct ELISA Kit

Kit Components

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. Anti-AGE Antibody (1000X) (Part No. 231701): One 20 µL vial of anti-AGE antibody.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 231702): 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.
8. Reduced BSA Standard (Part No. 233202): One 500 µL vial of 1 mg/mL reduced BSA in PBS.
9. AGE-BSA Standard (Part No. 231703): One 50 µL vial of 1 mg/mL AGE-BSA in PBS.

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 Reduced BSA and AGE-BSA Standards at -20ºC to avoid multiple freeze/thaw cycles. Store all other kit components at 4ºC.

Preparation of Reagents

- 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

1. Freshly prepare 10 µg/mL of Reduced BSA and AGE-BSA Standards by diluting the 1 mg/mL BSA standards in 1X PBS. Example: Add 20 µL to 1.980 mL of 1X PBS.
2. Prepare a series of AGE-BSA standards according to Table 1.

Standard Tubes	10 µg/mL AGE-BSA (µL)	10 µg/mL Reduced BSA (µL)	AGE-BSA (µg/mL)
1	200	200	5
2	160	240	4
3	120	280	3
4	80	320	2
5	40	360	1
6	20	380	0.5
7	10	390	0.25
8	0	400	0

Table 1. Preparation of AGE-BSA Standard Curve

Preparation of Sample Dilution

Samples containing high content of AGE protein adducts such as plasma or serum should be diluted 5-10 fold in 10 µg/mL Reduced BSA before used in the assay.
For example: 10 fold dilution of human plasma sample with Reduced BSA

1. Freshly prepare 10 µg/mL of Reduced BSA by diluting the 1 mg/mL BSA standard in 1X PBS. Example: Add 50 µL to 4.95 mL of 1X PBS.
2. Freshly prepare 10 µg/mL of human plasma sample by diluting the human plasma sample in 1X PBS. Example: Add 10 µL of 50 mg/mL human plasma sample to 50 mL of 1X PBS.
3. In a tube, mix 10 µg/mL of Reduced BSA and 10 µg/mL of human plasma sample at a ratio of 9:1. Example: Add 50 µL of 10 µg/mL of human plasma sample to 450 µL of 10 µg/mL of Reduced BSA.

Assay Protocol

1. Dilute unknown protein sample to 10 µg/mL in 1X PBS. Each protein sample and AGE-BSA Standard should be assayed in duplicate or triplicate.
2. Add 100 µL of the 10 µg/mL protein samples or prepared BSA standards to the 96-well Protein Binding Plate. Incubate at 37ºC for at least 2 hours or 4ºC overnight.

   Note: Lysate sample should not be prepared in lysis buffer containing Triton X-100, NP-40, or Igepal CA-630 because these detergents interfere with protein coating of the plate unless the

detergent concentration in the 10 µg/mL protein samples is no more than 0.001%. We recommend lysis by homogenization or sonication.

3. Wash wells 2 times with 250 µL 1X PBS per well. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess wash solution.
4. Add 200 µL of Assay Diluent per well and incubate for 1-2 hours at room temperature on an orbital shaker.
5. 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.
6. Add 100 µL of the diluted Anti-AGE Antibody to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 3 times according to step 5 above.
7. 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 5 above.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

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

Example of Results

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

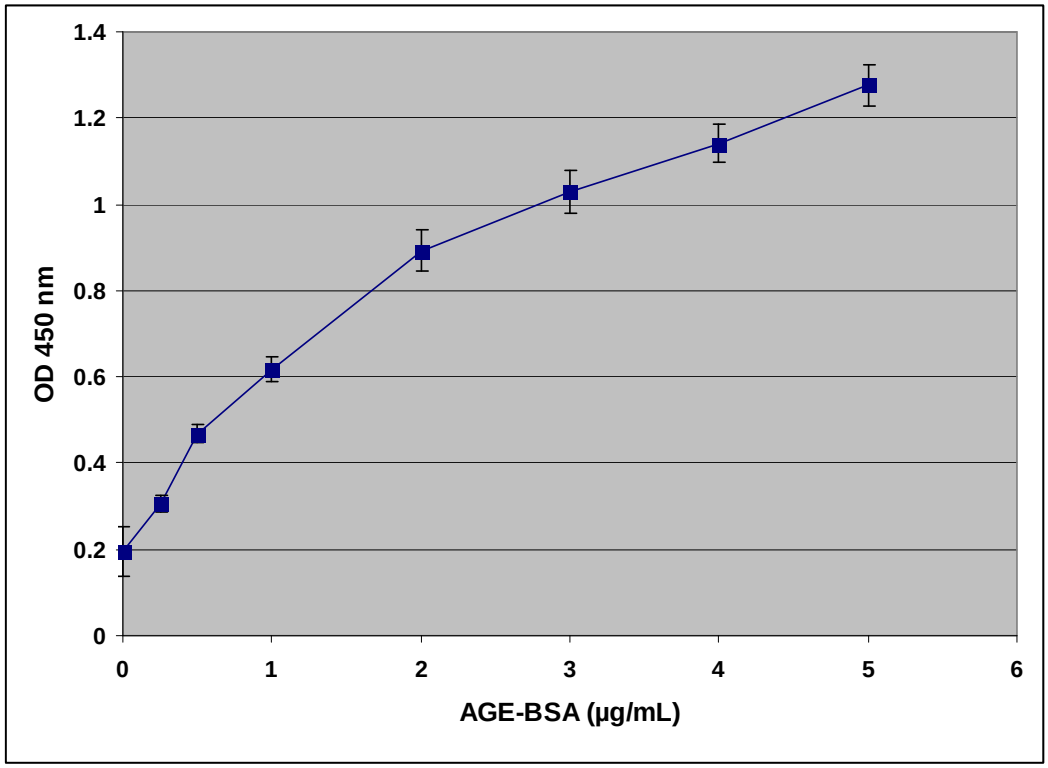

Figure 1: AGE-BSA ELISA Standard Curve.

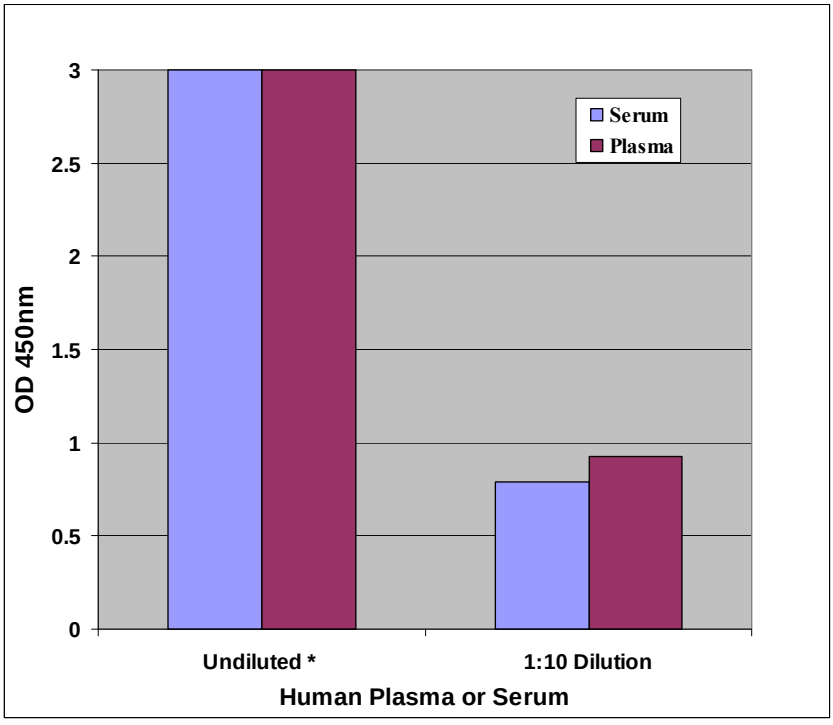

Figure 2: AGE level in human serum and plasma samples. The protein concentrations of normal human serum and plasma were first determined by Pierce BCA Protein Assay and subsequently dilute to 10 µg/mL with 1X PBS (Undiluted Sample) or further diluted 10-fold with Reduced BSA (1:10 Dilution). Samples were then coated onto a 96-well Protein Binding Plate. The AGE levels were determined as described in the Assay Protocol. **Undiluted plasma and serum samples result in saturation absorbance.*

References

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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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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com