Advanced Glycation End Product (AGE) ELISA Kit

Competitive Enzyme Immunoassay for the quantification of AGE protein adducts in purified protein, plasma, serum and cell lysate.

Catalog number: ARG81232

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INTRODUCTION

Advanced glycation end products (AGEs) are proteins or lipids that become glycated as a result of exposure to sugars. They can be a factor in aging and in the development or worsening of many degenerative diseases, such as diabetes, atherosclerosis, chronic kidney disease, and Alzheimer's disease.

AGEs affect nearly every type of cell and molecule in the body and are thought to be one factor in aging and some age-related chronic diseases. They are also believed to play a causative role in the vascular complications of diabetes mellitus.

Under certain pathologic conditions, such as oxidative stress due to hyperglycemia in patients with diabetes, and hyperlipidemia, AGE formation can be increased beyond normal levels. AGEs are now known to play a role as proinflammatory mediators in gestational diabetes as well.

The animal and human evidence is that significant amounts of dietary advanced glycation end-products (dAGEs) are absorbed, and that dAGEs contribute to the body's burden of AGE, and are associated with diseases such as atherosclerosis and kidney disease.

In the context of cardiovascular disease, AGEs can induce crosslinking of collagen which can cause vascular stiffening and entrapment of low-density lipoprotein particles (LDL) in the artery walls. AGEs can also cause glycation of LDL which can promote its oxidation. Oxidized LDL is one of the major factors in the development of atherosclerosis. Finally, AGEs can bind to RAGE (receptor for advanced glycation end products) and cause oxidative stress as well as activation of inflammatory pathways in vascular endothelial cells. [provide

from Wikipedia]

PRINCIPLE OF THE ASSAY

This is a Competitive Enzyme Immunoassay for the quantification Advanced Glycation End Product (AGE) in purified protein, plasma, serum and cell lysate. This Advanced Glycation End Product (AGE) ELISA Kit detects a variety of AGE structures including CML and pentosidine and it does not detect CEL or methylglyoxal (MG).

The AGE conjugate protein would be coated onto a microtiter plate. AGE-BSA standards or samples are then added to the AGE conjugate protein coated ELISA plate. After a brief incubation, an anti-AGE polyclonal antibody is added to bind the AGE-conjugate protein on the plate, AGE in the samples or AGE-BSA in the standard. After washing, any antibody unbounded on the plate would be wash way. Then HRP-conjugated secondary antibody is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of AGE-antibody complex present on the wells. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of Advanced Glycation End Product (AGE) in the sample is then determined by comparing the O.D of samples to the standard curve.

AGE-protein adducts in the samples or AGE-BSA in the standards compete with the AGE-coated plate for antibody binding. High AGE adduct content in a sample or high concentration AGE-BSA standard results in less AGE-antibody

binding complex on the plate, resulting in a low signal. Conversely, low AGE content in a sample or low concentration AGE-BSA standard result in most antibody binding to the AGE protein on the plate, producing a higher signal.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, aliquot and store the Anti-AGE Antibody, AGE-BSA Standard, AGE Conjugate and 100X Conjugate Diluent at -20°C to avoid repeated freeze-thaw cycles. Store all other kit components at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
96-well microplate	1 strips X 96 wells	4°C
10X Wash Buffer	100 ml	4°C
1000X Anti-AGE Antibody	10 µl	-20°C
1000X HRP-Conjugated Secondary Antibody	20 µl	4°C
AGE-BSA Standard (1 mg/ml)	125 µl	-20°C
AGE Conjugate (1 mg/ml)	50 µl	-20°C
100X Conjugate Diluent	300 µl	-20°C
Assay Diluent	50 ml (Ready-to-use)	4°C
TMB substrate	12ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: 620 nm as optional reference wave length)
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water
- 1X PBS.
- 1X PBS containing 0.1% BSA.
- Microplate shaker.
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, aliquot and store the Anti-AGE Antibody, AGE-BSA Standard, AGE Conjugate and 100X Conjugate Diluent at -20°C to avoid repeated freeze-thaw cycles. Store all other kit components at 4°C. Use the kit before expiration date.
- Briefly spin down the AGE-BSA Standard, Anti-AGE Antibody and HRP-Conjugated Secondary Antibody before use.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use.

Do not induce foaming.

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: Haemolytic and especially lipemic samples should not be used with this assay.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. Storage at 2-8°C.
- Anti-AGE Antibody and Secondary Antibody: Dilute the antibodies immediately before use, dilute the 1000X Anti-AGE antibody and 1000X HRP-Conjugated Secondary Antibody into Assay Diluent to yield 1X antibody working solutions. Do not store diluted solutions.
- 1X Conjugate Diluent: Dilute reagent immediately before use, dilute the 100X Conjugate Diluent into 1X PBS to yield 1X Conjugate Diluent. (e.g. Add 50 µl of 100X Conjugate Diluent to 4.95 ml of 1X PBS)
- 10 µg/ml AGE Conjugate: Dilute reagent immediately before use, diluting the 1.0 mg/ml AGE Conjugate into 1X PBS to yield AGE Conjugate working solution at a concentration of 10 µg/ml. (e.g. Example: Add 50 µl 1.0 mg/ml AGE Conjugate to 4.95 mL of 1X PBS)

  Note: for plate coating, 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.
- Sample: If the initial assay found samples contain AGE higher than the highest standard, the samples can be diluted with 0.1% BSA containing 1X PBS and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.
- AGE-BSA standard: Prepare a series dilution of AGE-BSA standards with Assay Diluent. The Assay Diluent serves as zero standard (0 µg/ml), and the rest of the standard serial dilution can be diluted with Assay Diluent as according to the suggested concentration table below:

Standard No	AGE-BSA concentration (µg/ml)	Assay Diluent (µl)	Standards (µl)
S1	100	360 µl	40 µl (1 mg/ml stock)
S2	50	200 µl	200 µl (S1)
S3	25	200 µl	200 µl (S2)
S4	12.5	200 µl	200 µl (S3)
S5	6.25	200 µl	200 µl (S4)
S6	3.125	200 µl	200 µl (S5)
S7	1.56	200 µl	200 µl (S6)
S8	0.78	200 µl	200 µl (S7)
S9	0.39	200 µl	200 µl (S8)
S0	0	200 µl	0 µl

Note: Dilutions for the standard curve and zero standard must be made and applied to the plate immediately. S0 serves as background.

ASSAY PROCEDURE

AGE Conjugate Protein Coated Plate:

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

1. Mix 10 µg/ml of AGE Conjugate and 1X Conjugate Diluent at 1:1 ratio.
2. Add 100 µl of the mixture from step1 to each well and incubate overnight at 4°C.
3. Remove the AGE Conjugate coating solution and wash twice with 1X PBS. After the last wash, remove any remaining 1X PBS by aspirating, decanting or blotting against clean paper towels.
4. 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.

Advanced Glycation End Product (AGE) ELISA procedure

1. Standards and samples should be assayed in duplicates.
2. Standard should be prepared immediately before use.
3. Add **50 µl of the AGE-BSA standards and samples** into the appropriate wells of the AGE Conjugate coated plate. Incubate for **10 minutes at room temperature** on a microplate shaker.
4. Add **50 µl of the 1:1000 diluted anti-AGE antibody** to each well, incubate for **1 hour at room temperature** on a microplate shaker
5. Aspirate each well and wash, repeating the process 2 times for a total **3 washes**. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **100 µ of the diluted HRP-Conjugated Secondary Antibody** to all wells and incubate for **1 hour at room temperature** on a microplate shaker.
7. **Warm TMB substrate solution** before next step. TMB substrate solution should be equilibrated to room temperature before use.
8. Aspirate each well and **wash** as step 5.
9. Add **100 µl of TMB substrate** solution into each well. Incubate for **2-20 mins at RT** on a microplate shaker. Avoid exposure to light.
   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
10. Add **100 µl of Stop Solution** to each well. Gently tap the plate to ensure thorough mixing. The color of the solution should change from blue to

yellow.

11. Read the OD with a microplate reader at 450nm immediately (optional: read at 620 nm as reference wave length).

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

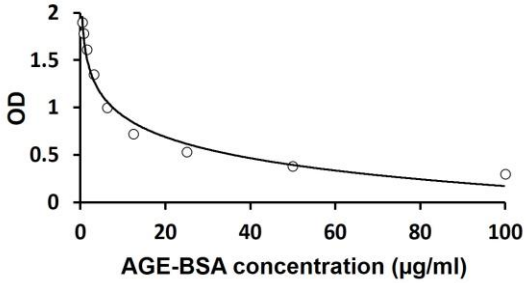
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

0.5 µg/ml

Assay Range

0.39- 100 µg/ml

Specificity

ARG81232 Advanced Glycation End Product (AGE) ELISA Kit detects a variety of AGE structures including CML and pentosidine and it does not detect CEL or methylglyoxal (MG).