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ab238539 AGE (Advanced Glycation End Products) Assay Kit

For the quantitative measurement of AGE in samples such as purified protein, plasma, serum and tissue and cell lysates.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

AGE (Advanced Glycation End Products) Assay Kit (ab238539) is designed for the rapid detection and quantitation of advanced glycation end product protein adducts.

Advanced Glycation End Products (AGE) are formed during the Maillard reaction where reducing carbohydrates react with lysine side chains and N-terminal amino groups of various macromolecules, particularly proteins. The advanced glycation end products can adversely affect the fuction of these macromolecules. One of the most prevalent advanced glycation end products, N-epsilon-(Carboxymethyl) Lysine, has been implicated in oxidative stress and vascular damage. The quantity of AGE adduct in protein samples is determined by comparing its OD with that of a known AGE-BSA standard curve.

2. Protocol Summary

Prepare all reagents, samples and standards as instructed.

Add 50 µL standard or sample to wells of AGE Conjugate coated plate and incubate for 10 minutes. Add 50 µL of the diluted anti-AGE antibody and incubate for 1 hour.

Washing steps with 250 µL 1X Wash Buffer.

Add 100 µL diluted Secondary Antibody-HRP Conjugate per well and incubate for 1 hour. Wash as before with 1X Wash buffer.

Add 100 µL of warm Substrate Solution and incubate for 2-20 minutes.

Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Read absorbance immediately on a microplate reader using 450 nm.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at +4°C immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage condition
Protein Binding Strip Well Plate	96 well	+4°C
Anti-AGE Antibody (1000X)	10 µL	-20°C
Secondary Antibody, HRP Conjugate (1000X)	20 µL	+4°C
Assay Diluent	50 mL	+4°C
10X Wash Buffer	100 mL	+4°C
Substrate Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
AGE-BSA Standard	125 µL	-20°C
AGE Conjugate	50 µL	-20°C
100X Conjugate Diluent	300 µL	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at O.D. 450 nm (620 nm as optional reference wave length)

6. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.
- Any components not listed here are ready to use as supplied.

6.1 AGE Conjugate Coated Plate

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

6.1.1 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.

6.1.2 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 of 1.0 mg/mL AGE Conjugate to 2.475 mL of 1X PBS and mix well.

6.1.3 Mix the 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.

6.1.4 Remove the AGE Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid.

6.1.5 Add 200 µL of Assay Diluent to each well and block for 1 hour at room temperature. Transfer the plate to 4°C and remove the Assay Diluent immediately before use.

6.2 1X Wash Buffer:

6.2.1 Dilute the 10X Wash Buffer Concentrate to 1X with deionized water.

6.2.2 Stir to homogeneity.

6.3 Anti-AGE Antibody and Secondary Antibody

6.3.1 Immediately before use dilute the Anti-AGE antibody 1:1000 and Secondary Antibody 1:1000 with Assay Diluent.

Δ Note: Do not store diluted solutions.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 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 as per the table below.

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

8. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.

9. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

9.1 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.

9.2 Add 50 µL of the diluted anti-AGE antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.

9.3 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.

9.4 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 9.3 above.

9.5 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.

Δ Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9.6 Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).

9.7 Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

10. Data Analysis

– Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

10.1 Average the duplicate reading for each standard, control and sample.

10.2 Plot the corrected values for each standard as a function of the final concentration of AGE Adduct.

10.3 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

10.4 Apply the corrected sample OD reading to the standard curve to get AGE Adduct µg/mL amount in the sample wells.

11. Typical Data

Typical standard curve - data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

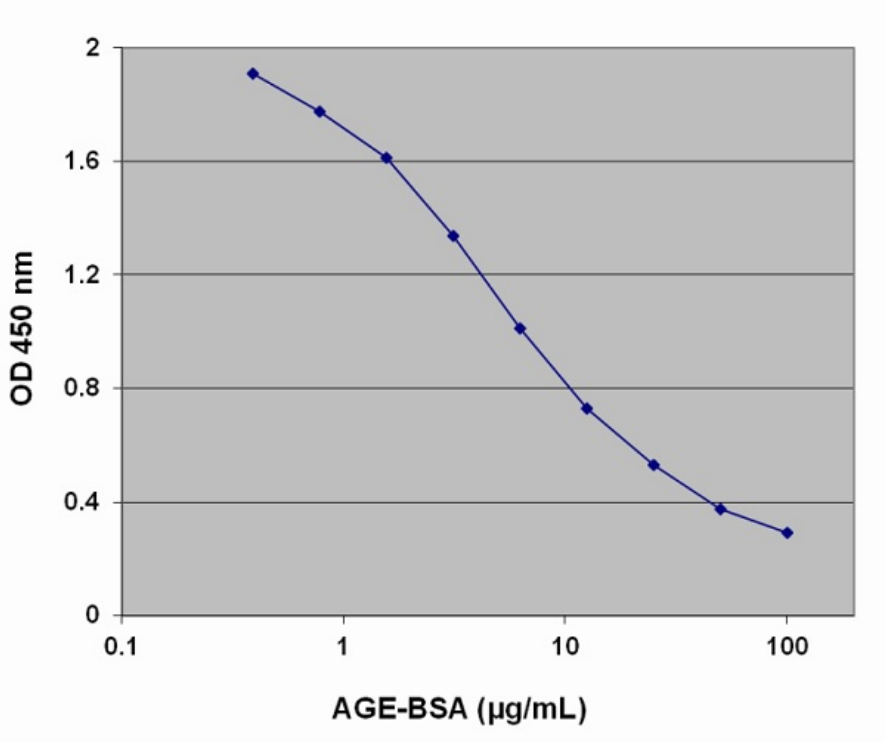

Figure 1. Typical Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

12. Species Reactivity

This kit is not species specific and can be used with samples from any species.

Please contact our Technical Support team for more information.

13. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)