

Version 5c, Last updated 12 March 2026

# ab273298

## Advanced Glycation End Products (AGEs) Assay Kit

View Kit datasheet: <https://www.abcam.com/ab273298>  
(use <https://www.abcam.cn/ab273298> for china, or  
<https://www.abcam.co.jp/ab273298> for Japan)

For the determination of AGE levels in biological fluids.

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

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## 1. Overview

Advanced Glycation End Products (AGEs) Activity Assay Kit (ab273298) can be used for the semi-quantitative estimation of fluorescent AGEs levels in Biological Fluids. The assay is based on the characteristic fluorescence (Ex/Em= 360/460 nm), a characteristic that is shared by almost all AGEs. The proprietary composition of the Assay Buffer specifically distinguishes between AGEs and non-oxidized proteins. The one-step assay uses oxidized Bovine Serum Albumin (AGE-BSA) as a Positive Control. BSA is used as Background Control and its fluorescence under assay conditions is defined as 1 relative fluorescence intensity in arbitrary units ( $AU$  or  $RFU_{\text{Sample}}/RFU_{\text{Background}}$ ).

## 2. Protocol Summary

Prepare all reagents as directed



Dilute serum and urine Samples to 1 mg/protein mL. For urine (optionally) measure Creatinine concentration



Add Positive Control, Samples and Background Control to appropriate wells and adjust volume to 200  $\mu$ L



Measure fluorescence (Ex/Em = 360/460 nm) in end-point mode.

### 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website <https://www.abcam.com/en-us>.

### 4. Storage and Stability

**Store kit at -20°C. Use within two months.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

## 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

<b>Item</b>	<b>Quantity</b>	<b>Storage temperature (before prep)</b>
Wash Buffer II	50 mL	-20°C
BSA Standard I	0.5 mL	-20°C
Positive Control Protein	500 µL	-20°C

PLEASE NOTE: Wash Buffer II was previously labelled as AGEs Assay Buffer, and Positive Control Protein as AGE Positive Control Protein and AGEs Positive Control (10 mg/ml), and BSA Standard I as BSA (50 mg/ml). The composition has not changed.

## **7. Materials Required, Not Supplied**

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

- Fluorescence microplate reader capable of measuring fluorescence at Ex/Em = = 360/460 nm
- 96-well white plate with flat bottom

## 8. Technical Hints

- **This kit is sold based on number of tests. A “test” simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

## 9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

### 9.1 **Wash Buffer II:**

Store at -20 °C. Bring to room temperature before use.

### 9.2 **BSA Standard I:**

Aliquot and Store at -20 °C. Bring to room temperature before use.

### 9.3 **Positive Control Protein:**

Aliquot and Store at -20 °C. Avoid repeated freeze-thaw cycles. During storage, Positive Control Protein may precipitate. Sonicate contents for short periods of time in order to resolubilize contents.

## 10. Sample Preparation

### 10.1 Serum:

- 10.1.1 Measure total protein concentration of Samples using BCA assay.
- 10.1.2 Dilute Samples to 1 mg/mL of protein using Wash Buffer II.
- 10.1.3 Add 4-10  $\mu$ L of diluted 1 mg/mL of Samples into wells of a white 96-well plate.
- 10.1.4 Adjust the volume to 200  $\mu$ L using Wash Buffer II in each well.

### 10.2 Urine:

- 10.2.1 Set an approximate protein concentration (1 mg/mL) of Sample(s) with Wash Buffer II.
- 10.2.2 Measure Creatinine concentrations of Samples using an appropriate kit.
- 10.2.3 Add 4-10  $\mu$ L of urine Samples(s) at 1 mg/ml protein concentration into wells of a white 96-well plate and label as "Sample".
- 10.2.4 Adjust the volume to 200  $\mu$ L using Wash Buffer II in each well.

### 10.3 Background control:

- 10.3.1 Prepare 1 mg/mL BSA Standard I by adding 2  $\mu$ L of 50 mg/mL BSA Standard I into 98  $\mu$ L Wash Buffer II.
- 10.3.2 Add 4-10  $\mu$ L (same volume as "Samples") of 1 mg/ml BSA Standard I into wells and label as "Background Control".
- 10.3.3 Adjust the volume to 200  $\mu$ L using Wash Buffer II in each well.

### 10.4 Positive control:

- 10.4.1 Prepare 1 mg/mL Positive Control Protein by adding 2  $\mu$ L of 10 mg/mL Positive Control Protein into 18  $\mu$ L Wash Buffer II.
- 10.4.2 Add same volume of 1 mg/mL Positive Control Protein into wells as "Positive Control".
- 10.4.3 Adjust the volume to 200  $\mu$ L using Wash Buffer II in each well

$\Delta$  **Note:** Urine: it is recommended to measure Creatinine concentration in samples.

$\Delta$  **Note:** The estimation of AGEs is the result of ratios between samples and background. Therefore, it is critical to maintain the added volume (or mass) of all Samples (Serum and/or Urine), Background Control and Positive Control the same (4 -10  $\mu$ L).

## 11. Standard Curve of AGE and BSA (Optional):

- 11.1 **AGE:** Prepare a 1 mg/mL (1 µg/µL) solution of Positive Control Protein by diluting 40 µL of 10 mg/mL Positive Control Protein with 360 µL of Wash Buffer II.
- 11.2 **BSA Standard I:** Prepare a 1 mg/mL (1 µg/µL) solution of BSA Standard I by diluting 8 µL of 50 mg/mL BSA Standard I with 392 µL of Wash Buffer II.
- 11.3 Add 0, 20, 40, 60, 80, 100 µL of 1 mg/mL (1 µg/µL) Positive Control Protein or 1 mg/ml (1 µg/µl) BSA Standard I into a series of wells in a 96-well white plate and adjust the final volume to 200 µL/well with Wash Buffer II to generate 0, 20, 40, 60, 80 and 100 µg/well of AGE-BSA or BSA Standard I. Mix well

Standard #	10 mg/ml AGE-Standard (µL)	Wash Buffer II (µL)	AGE (µg/well)
1	100	100	100
2	80	120	80
3	60	140	60
4	40	160	40
5	20	180	20
6	0	200	0

- 11.4 Mix well and measure the fluorescence (Ex/Em = 360/460 nm) in end point mode.

## 12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

- 12.1 Prepare Standards as described in Section 11.
- 12.2 Prepare Samples, Background Control and Positive Control as described in Section 10.
- 12.3 Incubate the plate at room temperature for 5 minutes, protected from light.
- 12.4 Measure fluorescence (Ex/Em= 360/460 nm) at room temperature in end point mode using a fluorescence microplate reader.

### 13. Calculations

13.1 For calculation purposes, the Fluorescence (Ex/Em=360/460 nm) of the Background Control (1 mg/mL BSA Standard I) is used as a reference and defined as 1 arbitrary unit (AU).

$$AGEs \text{ amount in serum} = \frac{RFU \text{ sample}}{RFU_{background} * mg \text{ of serum protein in wells}}$$

=AU/mg of serum protein

Δ Note: based on the protocol, the assay is run with 0.004-0.01 mg of protein/ well since 4-10 μL of 1 mg/ml serum protein is added

$$AGEs \text{ amount in serum} = \frac{RFU \text{ sample}}{RFU_{background} * C * V}$$

= AU/mg Urine Creatinine

V= volume of urine at 1 mg/ml protein added to well in ml  
C= concentration of creatinine (mg/ml) in 1 mg/ml protein sample  
RFUsample= RFU generated by sample (Serum or Urine; 1 mg/ml)  
RFUbackground Control= the RFU generated by BSA Standard I (1 mg/ml)

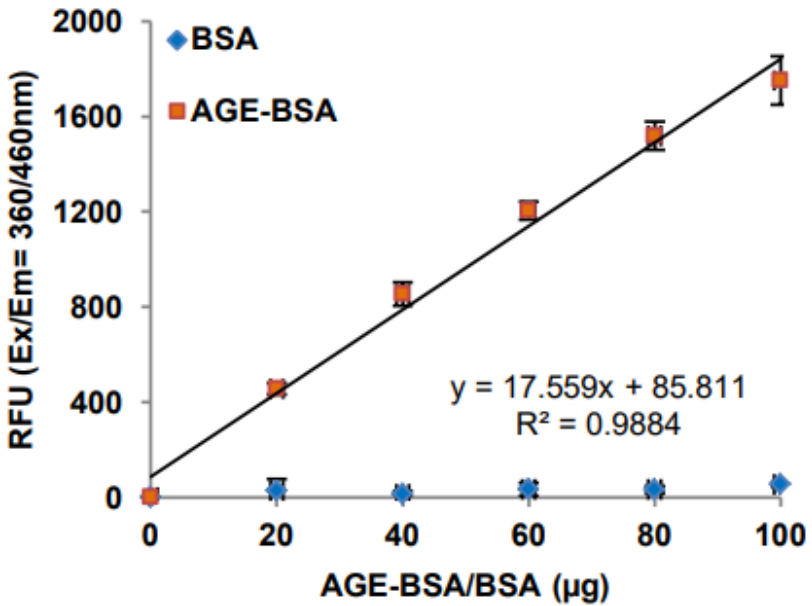
Δ Note: If output of your creatinine concentration assay is in mM (or μmol/ml) it can be converted using the molecular weight of creatinine, or AGEs can be normalized to nmol of urine creatinine. Creatinine molecular weight: 113.12 g/mol

#### Unit definition:

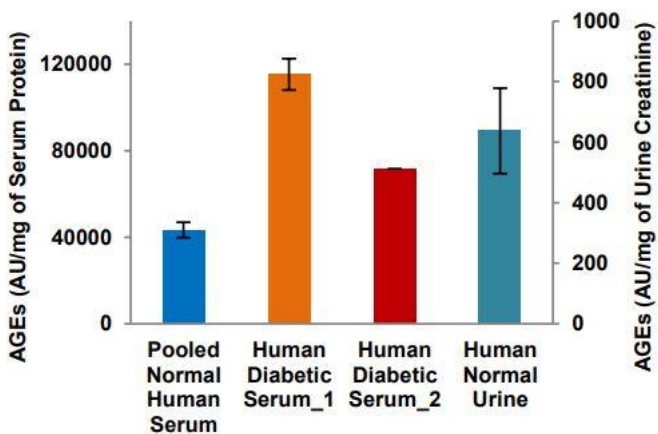
AGEs are expressed as arbitrary units (AU) relative to serum proteins for serum samples and urine creatinine for urine samples.

## 14. Typical Data

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



**Figure 1.** AGEs-Positive (AGE-BSA) fluorescence versus BSA. The assay can distinguish both species (AGEs and non-oxidized proteins).



**Figure 2.** AGEs amount in Pooled Human Serum from healthy donors (4  $\mu$ g serum protein), Human Diabetic Serum (4  $\mu$ g serum protein) and Human Urine from healthy donor.

## 15. FAQ / Troubleshooting

General troubleshooting points are found at

<https://www.abcam.com/en-us/products/biochemical-assays>.

## 16. Notes

## Technical Support

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