

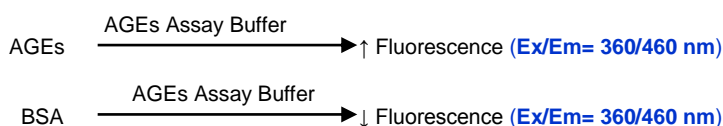
# Advanced Glycation End Products (AGEs) Assay Kit

7/18

(Catalog # K929-100; 100 assays; Store at -20°C)

## I. Introduction:

Advanced Glycation End Products (AGEs; also termed as glycotoxins) are a group of heterogeneous compounds formed through non-enzymatic glycation and oxidation processes between reducing sugars and protein side chains, lipids, or nucleic acids. Initial glycation and oxidation processes usually form Schiff bases and Amadori products. Glycation itself causes molecular rearrangements that lead to the generation of AGEs. The formation of these molecules is part of normal metabolism; however, under certain pathological conditions, such as oxidative stress, the production of AGEs can be abnormally excessive, leading to pathogenic conditions. The formation and accumulation of AGEs have been implicated in aging and in the development of many degenerative diseases, such as diabetes, chronic kidney disease, atherosclerosis and Alzheimer's disease. By definition, there is not a universally accepted method to measure or quantify AGEs due to their heterogeneity. Nevertheless, since most AGEs have intrinsic fluorescence, measurements of AGE-specific fluorescence may serve as a simple and useful test to monitor circulating AGEs levels and monitor AGEs excretion. BioVision's Advanced Glycation End Products (AGEs) Assay Kit is a 96-well microplate-based assay that 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  $RFU_{\text{Sample}}/RFU_{\text{Background}}$ ).



## II. Applications:

- Measurement of AGEs levels in Biological Fluids

## III. Sample Type:

- Serum, Urine

## IV. Kit Contents:

Components	K929-100	Cap Code	Part Number
AGEs Assay Buffer	50 ml	NM	K929-100-1
BSA (50 mg/ml)	500 µl	Yellow	K929-100-2
AGEs Positive Control (10 mg/ml)	500 µl	Red	K929-100-3

## V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer

## VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C. Briefly centrifuges small vials prior to opening. Read entire protocol before performing the assay. Use within two months.

- **AGEs Assay Buffer:** Store at 4 °C. Bring to room temperature before use.
- **BSA:** Aliquot and Store at -20 °C. Bring to room temperature before use.
- **AGEs Positive Control:** Aliquot and Store at -20 °C. Avoid repeated freeze-thaw cycles. During storage, AGEs Positive Control may precipitate. Sonicate contents for short periods of time in order to resolubilize contents.

## VII. AGEs Assay Protocol:

### 1. Sample Preparation:

**Serum:** Measure total protein concentration of Sample(s) using BCA assay (BioVision Cat# K818 or equivalent). Dilute Sample(s) to 1 mg/ml of protein using AGEs Assay Buffer. **Record Dilution Factor.** Add 4-10 µl of diluted 1 mg/ml of Sample(s) into wells of a white 96-well plate and label as "Sample".

**Urine:** Set an approximate protein concentration (~1 mg/ml) of Sample(s) with AGEs Assay Buffer. **Record Dilution Factor.** Measure Creatinine concentration(s) of Sample(s) (BioVision Cat# K625-100). Add 4-10 µl of 1 mg/ml of Sample(s) into wells of a white 96-well plate and label as "Sample".

**Background Control:** Prepare 1 mg/ml BSA by adding 2 µl of 50 mg/ml BSA into 98 µl AGEs Assay Buffer. Add volume (4-10 µl, same as "Samples") of 1 mg/ml BSA into wells and label as "Background Control".

**Positive control:** Prepare 1 mg/ml AGEs Positive Control by adding 2 µl of 10 mg/ml AGEs Positive Control into 18 µl AGEs Assay Buffer. Add same volume of 1 mg/ml AGEs Positive Control into wells as "Positive Control".

Adjust the volume to 200 µl using AGEs Assay Buffer in each well including Sample(s), Background Control and Positive Control, Mix well.

FOR RESEARCH USE ONLY!

**Notes:**

- a. *Urine:* it is recommended to measure Creatinine concentration in samples (BioVision Cat# K625-100: Creatinine Colorimetric/Fluorometric Assay Kit).
  - b. **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 µl).**
- 2. AGE and BSA Standard Curves (Optional):** **AGE:** Prepare a 1 mg/ml (1 µg/µl) solution of AGEs Positive Control by diluting 40 µl of 10 mg/ml AGEs Positive Control with 360 µl of AGEs Assay Buffer. **BSA:** Prepare a 1 mg/ml (1 µg/µl) solution of BSA by diluting 8 µl of 50 mg/ml BSA with 392 µl of AGEs Assay Buffer. Add 0, 20, 40, 60, 80, 100 µl of 1 mg/ml (1 µg/µl) AGEs-Positive Control or 1 mg/ml (1 µg/µl) BSA into a series of wells in a 96-well white plate and adjust the final volume to 200 µl/well with AGEs Assay Buffer to generate 0, 20, 40, 60, 80 and 100 µg/well of AGE-BSA or BSA. Mix well.
- 3. Measurement:** Incubate the plate at room temperature for 5 minutes, protected from light. Measure fluorescence (Ex/Em= 360/460 nm) at room temperature in end point mode using a fluorescence microplate reader.
- 4. Calculation:** For calculation purposes, the Fluorescence (Ex/Em=360/460 nm) of the Background Control (1 mg/ml BSA) is used as a reference and defined as 1 arbitrary unit (AU).

$$\text{AGEs amount in Serum} = \frac{[\text{RFU, Sample}] \cdot \text{D}}{[\text{RFU, Background Control}] \cdot [\text{mg of serum protein in wells}]} = \text{AU/mg of Serum Protein}$$

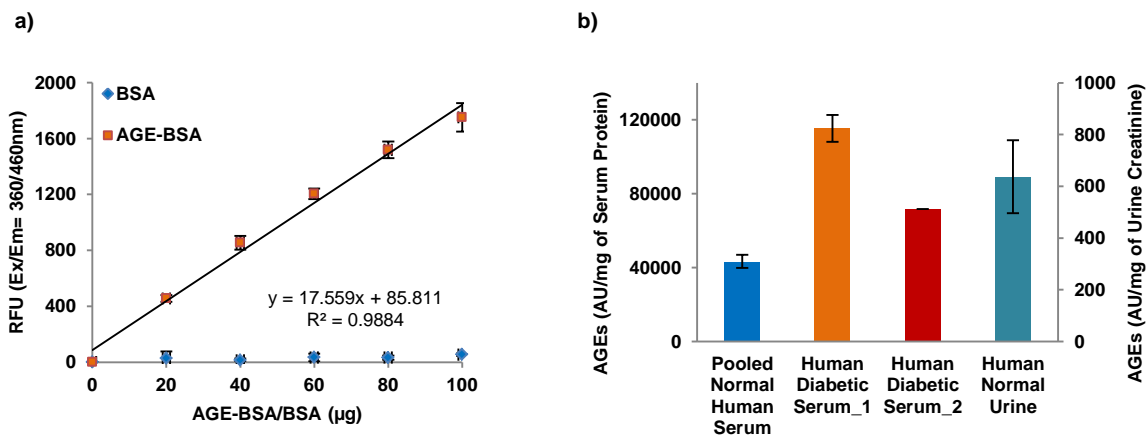
$$\text{AGEs amount in Urine} = \frac{[\text{RFU, Sample}] \cdot \text{D}}{[\text{RFU, Background Control}] \cdot [\text{mg of urinary creatinine in wells}]} = \text{AU/mg of Urine Creatinine}$$

Where: D is Dilution Factor

RFU, Sample is the RFU generated by sample (Serum or Urine; 1 mg/ml)

RFU, Background Control is the RFU generated by BSA (1 mg/ml)

**Note:** AGEs are expressed as arbitrary units (AU) corrected by serum proteins for serum samples and urine creatinine for urine samples.



**Figure:** (a) AGEs-Positive (AGE-BSA) fluorescence versus BSA. The assay can distinguish both species (AGEs and non-oxidized proteins). (b) AGEs amounts in Pooled Human Serum from healthy donors (4 µg serum protein), Human Diabetic Serum (4 µg serum protein) and Human Urine from healthy donor. Assays were performed following the kit protocol.

**VIII. RELATED PRODUCTS:**

- Methylglyoxal Assay Kit (Colorimetric) (K500)
- PicoProbe™ Methylglyoxal Assay Kit (Fluorometric) (K461)
- Biotinylated AGE-BSA (7929)
- Biotinylated Glucose AGE-BSA-II (7930)
- Advanced Glycation End product (AGE)-BSA (2221)
- Glucose AGE-BSA-II (2223)