



Mild-AGE-BSA

Product Description	Fatty acid-free bovine serum albumin (BSA) (0.05 g/ml) was incubated with 50 mM of glucose in a 0.05 M sodium phosphate buffer (pH 7.4) at 37°C for 24 weeks, followed by dialysis against PBS. The CML content (0.4 mol CML/mol BSA) was determined by amino acid analysis. This method is prepared mildly-modified-AGE-BSA (mild-AGE-BSA). The CML content for the mild-AGE-BSA is 24.4 mmol CML/mol Lys. However, the CML contents for the diabetic (DM)- and non-diabetic human lens samples were about 17.4 mmol /mol Lys and about 8.6 mmol/mol Lys, respectively, thus indicating that the CML content of mild-AGE-BSA was similar to physiological samples.
Volume	200 ul
Concentration	1 mg/ml
Storage	Store below -20°C (below -70°C for prolonged storage). Aliquot to avoid cycles of freeze/thaw.

- References**
1. Nagai R., Mera K, Nakajou K., Fujiwara Y., Iwao Y., Imai H., Murata T and Otagiri M. The ligand activity of AGE-proteins to scavenger receptors is dependent on their rate of modification by AGEs. *Biochim Biophys Acta - Molecular Basis of Disease*. 1772, 1192-1198 (2007) PMID: [18053692](https://pubmed.ncbi.nlm.nih.gov/18053692/)

Characterization

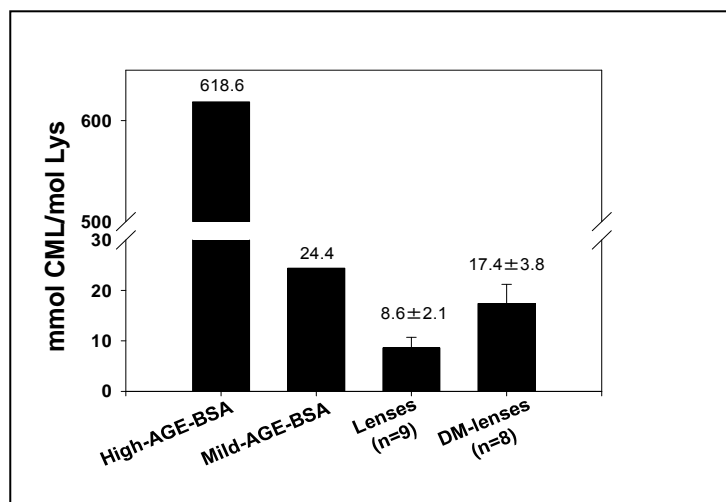


Fig.1 Comparison of CML content in the samples by HPLC

ELISA protocol

Coating

- 1) Distribute 100 ul / well of the sample solution (1 ug/mL in PBS) to 96 well microtiter plates (Thermo, MaxiSorp).
- 2) Incubate the plates 2h at RT or overnight at 4 degrees.
- 3) Discard the supernatant of sample solution.
- 4) Wash the plates three times with washing.buf.(PBS/0.05%Tween 20)

Blocking

- 1) Distribute 200 ul / well of 0.5% gelatin-PBS to 96 well microtiter plates
- 2) Incubate the plates 1h at RT.
- 3) Discard the the supernatant of 0.5% gelatin-PBS
- 4) Wash the plates three times with washing.buf.(PBS/0.05%Tween 20)

Primary antibody

- 1) Distribute 100 ul / well of Primary antibodies diluted with washing buf. as suggested in the APPLICATIONS to each well.
- 2) Incubate the plates 1h at RT.
- 3) Discard the supernatant of Primary antibody solution.
- 4) Wash the plates three times with washing.buf.(PBS/0.05%Tween 20)

Secondary antibody

- 1) Distribute 100 ul / well of secondary antibodies (HRP-anti mouse IgG) diluted with washing buf. as suggested in the APPLICATIONS to each well.
- 2) Incubate the plates 1h at RT.
- 3) Discard the supernatant of secondary antibody.
- 4) Wash the plates three times with washing.buf.(PBS/0.05%Tween 20)

OPD color reaction

- 1) Reaction for 2-10 minutes at RT.
- 2) Distribute 100 uL / well of 2M H₂SO₄ to each well and stop enzyme reaction.
- 3) After gentle mixing, determine the absorbance at 492 nm of each well by a spectrophotometer.

RELATED PRODUCTS:

Product Name	Quantity	Maker	Cat#
Anti N ^F -(carboxymethyl) lysine [CML] (2G11) Monoclonal Antibody	100 ul	CAC	AGE-M01
Anti N ^F -(carboxyethyl) lysine [CEL] (CEL-SP) Monoclonal Antibody	100 ul	CAC	AGE-M02
Anti GA-pyridine (2A2) Monoclonal Antibody	100 ul	CAC	AGE-M03
Anti N ^ω -(carboxymethyl) arginine [CMA] (3F5) Monoclonal Antibody	100 ul	CAC	AGE-M04
CML-BSA/Nε-(carboxymethyl) lysine-BSA	200 ul	CSR	AGE-GP01
CEL-BSA/Nε-(carboxyethyl) lysine-BSA	200 ul	CSR	AGE-GP02
GA-BSA/Glycolaldehyde-BSA	200 ul	CSR	AGE-GP03
Ribose-gelatin	500 ul	CSR	AGE-GP04
Mild-AGE-BSA	200 ul	CSR	AGE-GP05

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