COSMO BIO CO., LTD.
Inspiration for Life Science

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Catalog No. AGE-GP05

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

Characterization

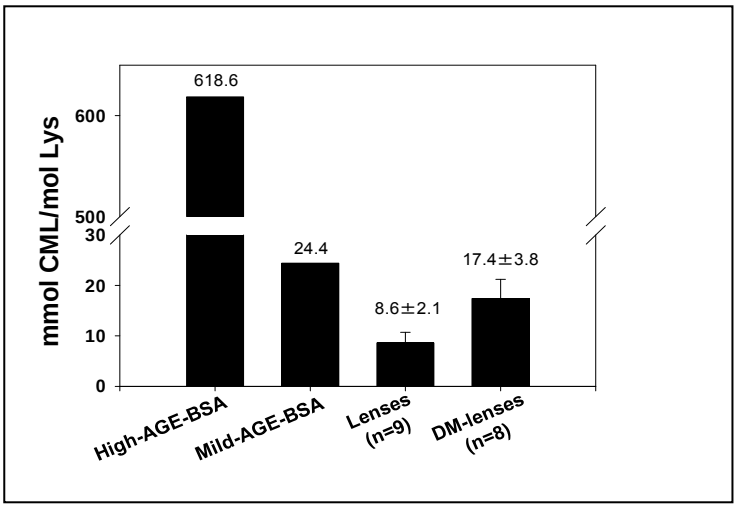

Fig.1 Comparison of CML content in the samples by HPLC

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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 H_2SO_4 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^{ε}-(carboxymethyl) lysine [CML] (2G11) Monoclonal Antibody	100 ul	CAC	AGE-M01
Anti N^{ε}-(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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