

For research use only. Not for clinical diagnosis

Catalog No. AGE-GP02

CEL-BSA/N^ε-(carboxyethyl) lysine-BSA

Product Description	Bovine serum albumin (BSA) (50 mg/ml) was incubated at 37°C for 24 h with pyruvate and 100 mM sodium cyanoborohydride in 0.2 M sodium phosphate buffer (pH 7.8), followed by dialysis against PBS. The CEL content (2.6 mol CEL/mol BSA) was determined by amino acid analysis.			
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	 Nagai R., Fujiwara Y., Mera K., Yamagata K., Sakashita N., Takeya M. Immunochemical detection of N^ε-(carboxyethyl)lysine using a specific antibody. J. Immunol. Methods 332, 112-120 (2008) PMID: <u>18242632</u> Koito W., Araki T., Horiuchi S., Nagai R. Conventional antibody against N^ε-(Carboxymethyl)lysine (CML) shows cross-reaction to N^ε-(Carboxyethyl)lysine (CEL): Immunochemical quantification of CML with a specific antibody. J Biochem. 136, 831-837 (2004) PMID: <u>15671494</u> Nagai R., Araki T., Hayashi CM., Hayase F., Horiuchi S Identification of N^ε-(carboxyethyl)lysine, one of the methylglyoxal-derived AGE structures, in glucose-modified protein: mechanism for protein modification by reactive aldehydes. J Chromatogr B Analyt Technol Biomed Life Sci. 788, 75-84 (2003) PMID: <u>12668073</u> 			

Characterization



Fig.1 Immunoreactivity of CEL(CEL-SP) monoclonal antibody to CEL-BSA and CML-BSA

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ELISA protocol

Coating

- 1) Distribute 100 µl / 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 µl / 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 μl / 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 μl / 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 μ L / 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 ^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/Nɛ-(carboxymethyl) lysine-BSA	200 ul	CSR	AGE-GP01
CEL-BSA/Nɛ-(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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