

Catalog No. AGE-GP01

CML-BSA/N^ε-(carboxymethyl) lysine-BSA

Lot#: A-001

Product
Description
(Lot#: A-001)

Bovine serum albumin (BSA) (50 mg/ml) was incubated at 37°C for 24 h with glyoxylic acid and 100 mM sodium cyanoborohydride in 0.2 M sodium phosphate buffer (pH 7.8), followed by dialysis against PBS. The CML content (2.3 mol CML/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 Nε-(carboxyethyl)lysine using a specific antibody.
 J. Immunol. Methods 332, 112-120 (2008) PMID: 18242632
- Koito W., Araki T., Horiuchi S., Nagai R. Conventional antibody against Nε-(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: 15671494
- 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: 12668073

Characterization

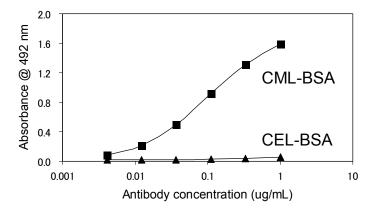


Fig.1 Immunoreactivity of the CML(2G11) monoclonal antibody to CML-BSA and CEL-BSA

ELISA protocol

Coating

- 1) Distribute 100 µl / well of the sample solution (1 µg/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 μ l / 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 lgG) 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/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 |

For research use only, Not for diagnostic use.



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Catalog No. AGE-GP01

CML-BSA/N^ε-(carboxymethyl) lysine-BSA

Lot#: A-002

Product
Description
(Lot#: A-002)

Bovine serum albumin (BSA) (50 mg/ml) was incubated at 37°C for 24 h with glyoxylic acid and 100 mM sodium cyanoborohydride in 0.2 M sodium phosphate buffer (pH 7.8), followed by dialysis against PBS. The CML content (2.58 mol CML/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 Nε-(carboxyethyl)lysine using a specific antibody.
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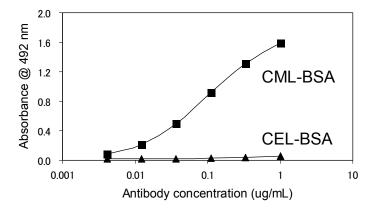


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- 4) Wash the plates three times with washing.buf.(PBS/0.05%Tween 20)

Secondary antibody

- 1) Distribute 100 μ I / 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)

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- 1) Reaction for 2-10 minutes at RT.
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RELATED PRODUCTS:

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|---|----------|-------|----------|
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| 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 |
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| Mild-AGE-BSA | 200 ul | CSR | AGE-GP05 |

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