ANTI N°-(carboxymethyl) lysine (CML)

BACKGROUND
N°-(carboxymethyl) lysine (CML) is reported as a major antigenic AGE structure. Recent studies demonstrate that CML is generated by the oxidative cleavage of Amadori products by hydroxyl radical, peroxynitrite and hypochlorous acid, thus suggesting CML to be an important biological marker of oxidative stress in vivo.

Product type: Primary antibody
Immunogen: CML-HSA
Host Species: Mouse
Fusion Partner: P3U1
Clone Designation: 2G11
Isotype: IgG1
Host: Mouse
Source: Ascites
Purification: Protein G
Form: Liquid
Formulation Buffer: PBS containing 0.1% proclin and 2% sorbitol as a preservative
Concentration: 0.2 mg/ml
Volume: 100 ul
Label: Unlabeled
Specificity: CML
Cross species reactivity: -
Storage: Store below -20°C (below -70°C for prolonged storage) Aliquot to avoid cycles of freeze/thaw.

Application notes
Recommended dilutions
Western blotting: 1/200 - 1/400
Immunofluorescence: 1/100 - 1/200
ELISA: 1/200 - 1/400

Other applications have not been tested.
Optimal dilutions/concentrations should be determined by the end user.

References

www.cosmobio.co.jp
ANTIBODY CHARACTERIZATION

Fig. 1  CML production pathway

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

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Anti- CML monoclonal antibody Cat#: AGE-M01

Version#: 3-190117
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 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:

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Quantity</th>
<th>Maker</th>
<th>Cat#</th>
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<tbody>
<tr>
<td>Anti Nε-(carboxymethyl)lysine [CML] (2G11) Monoclonal Antibody</td>
<td>100 ul</td>
<td>CAC</td>
<td>AGE-M01</td>
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<td>100 ul</td>
<td>CAC</td>
<td>AGE-M02</td>
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