

#### MONOCLONAL ANTIBODY

For research use only. Not for clinical diagnosis

#### Catalog No. AGE-M03

# Anti GA-pyridine

#### BACKGROUND

Glycolaldehyde formed as a result of the myeloperoxidase- $H_2O_2$  (MPO) reaction can react with proteins to yield various AGEs. Recently, a novel specific GA-derived AGE, called GA-pyridine, has been described in foam cells and the extracellular matrix of human atherosclerotic fibrotic lesions, glomerular mesangial and Bruch's membrane and choroid.

Product type	Primary antibody			
Immunogen	GA-LDL			
Host Species	Mouse			
Fusion Partner	P3U1			
Clone Designation	2A2			
Isotype	lgG1			
Host	Mouse			
Source	Ascites			
Purification	Protein G			
Form	Liquid			
Formulation Buffer	PBS containing 0.1% proclin as a preservative			
Concentration	0.2 mg / ml			
Volume	100 ul			
Label	Unlabeled			
Specificity	GA-pyridine			
Cross species reactivity	- · · · · · · · · · · · · · · · · · · ·			
Storage	Store below -20 $^\circ\!{\rm C}$ (below -70 $^\circ\!{\rm C}$ for prolonged storage) Aliquot to avoid cycles of freeze/thaw.			
Application notes Recommended dilutions	<ul> <li>Western blotting: 1/200 - 1/2000</li> <li>Immunofluorescence: 1/200 - 1/400</li> <li>ELISA: 1/200 - 1/400</li> </ul>			
	Other applications have not been tested. Optimal dilutions/concentrations should be determined by the end user.			
References	<ol> <li>Nagai R., Hayashi CM., Xia L., Takeya M., Horiuchi S: Identification in human atherosclerotic lesions of GA-pyridine, a novel structure derived from glycolaldehyde-modified proteins. J Biol Chem. 277, 48905-48912 (2002) PMID: <u>12377783</u></li> <li>Glenn JV., Mahaffy H., Wu K., Smith G., Nagai R., Simpson DAC., Boulton ME., Stitt AW. Advanced Glycation End Product (AGE) Accumulation on Bruch's Membrane:</li> </ol>			
	LINKS TO AGE-RELATED KPE Dystunction. Invest. Ophth. Vis. Sci. 50, 441-451 (2009) PMID: 18676633			

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Fig.1 GA-pyridine structure



Fig.2 Immunoreactivity of the GA-pyridine (2A2) monoclonal antibody to GA-HAS, GA-BSA, MG-HAS, GO-HSA and HSA

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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 <sup>ε</sup> -(carboxymethyl) lysine [CML] (2G11) Monoclonal Antibody	100 ul	CAC	AGE-M01
Anti N <sup>ε</sup> -(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 <sup><math>\omega</math></sup> -(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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