CML-HSA/N$^{\varepsilon}$-(Carboxymethyl)lysine-HSA

Cat# CY-R2066

Lot No.
Sterile condition
200 µg (1 mg/mL x 200 µL)

Background: Reducing sugars react with protein amino groups to form a diverse group of protein-bound moieties with fluorescent and cross-linking properties. These compounds, called advanced glycosylation end products (AGEs), have been implicated in the structural and functional alterations of proteins that occur during aging and long-term diabetes. Although several AGE structures have been reported (1, 2), it was demonstrated that N$^{\varepsilon}$-(Carboxymethyl)lysine (CML) is a major antigenic AGE structure. CML concentration is also increased in patients who have diabetes with complications, including nephropathy (3–5), retinopathy (6), and atherosclerosis (7–9). CML is also recognized by receptor for AGE (RAGE), and CML-RAGE interaction activates cell signaling pathways such as NF-B and enhances the expression of vascular cell adhesion molecule-1 in human umbilical vein endothelial cells (10).

Product Description: Prepared according to the method described in Ikeda K et al. (1). Approx. 14 mole of CML/1 mole of HSA.

Product Size: 1 mg/mL x 200 µL

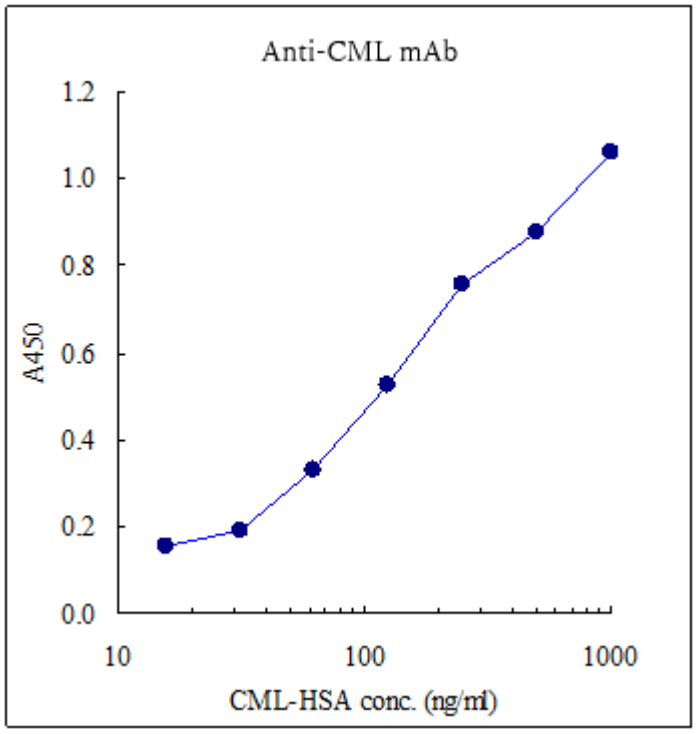
Formulation: Supplied frozen in a buffer containing 10 mM PBS (pH 7.2).

Specificity: Detected by anti-N$^{\varepsilon}$-(Carboxymethyl)lysine antibody.

Recommended concentration: Coating microtiter plate for competitive ELISA: 500-1,000 ng/mL.

Storage and Stability: Stable for 12 months at -20°C from date of shipment. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap. Aliquot CML-HSA to avoid repeated freezing and thawing.

Fig.1 Direct ELISA for testing the reactivity of anti-CML/N$^{\varepsilon}$-(Carboxymethyl)lysine monoclonal antibody (a component of the kit product [Cat#CY-8066]) against CML-adduct in CML-HSA (CY-R2066)



Direct ELISA Protocol:

Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

Coating Buffer: 25 mM Sodium carbonate buffer (pH 9.0)

Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20

Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).

10X TBS (Tris-buffered saline): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.0 with HCl (use at 1X).

Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20. 0.05 % NaN_3 with 2 % BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 0.4 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%) and 100 µl of 10 % NaN_3.

Secondary Antibody Dilution Buffer: 1X TBS, with 2 % BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 0.4 g BSA and mix well.

Secondary Antibody: anti-mouse IgG antibody conjugated to horseradish peroxidase (HRP).

HRP Detection: 1 mM 3, 3', 5, 5'–tetramethhylbenzidine 2HCl/TMB (KPL, Inc.) and 10 mM H_2O_2 in 20 mM Sodium citrate buffer (pH 5.5).

Stop Solution: 0.5 N H_2SO_4

ELISA Procedure

1. Make serial dilutions of CML-HSA, CY-R2052 (0.5 to 256 ng/ml) or sample.
2. Add 100 µl of diluted sample to each well in 96-well microtiter plate and incubate overnight at 4°C.
3. Wash 2 times with Wash Buffer.
4. Add 200 µl of Blocking Buffer to each well and incubate for 1.5 h at 37C or overnight at 4°C.
5. Wash 4 times with Wash Buffer.
6. Add 100 µl of anti-N$^{\varepsilon}$-(Carboxymethyl)lysine /CML monoclonal antibody and incubate for 1 hr at room temperature.
7. Wash wells five times with Wash Buffer making sure each well is filled completely. Remove residual Wash Buffer by gentle tapping or aspiration.
8. Add 100 µl of anti-mouse IgG antibody conjugated to horseradish peroxidase (HRP) and incubate for 1 hr at room temperature.
9. Wash wells five times with Wash Buffer making sure each well is filled completely. Remove residual Wash Buffer by gentle tapping or aspiration.
10. Add 100 µL of Substrate Reagent to each well and incubate at room temperature for 5–15 minutes.
11. Add 100 µL of Stop Solution to each well in the same order as the previously added Substrate Reagent.
12. Measure absorbance in each well using a spectrophotometric plate reader at dual wavelengths of 450/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. Read the plate at 450 nm if only a single wavelength can be used. Wells must be read within 30 minutes of adding the Stop Solution.

References:

1. Ikeda K, Higashi T, Sano H, Jinnouchi Y, Yoshida M, Araki T, Ueda S, Horiuchi S: *Biochemistry* 35: 8075 –8083,1996
2. Reddy S, Bichler J, Wells-Knecht KJ, Thorpe SR, Baynes JW: *Biochemistry* 34: 10872 –10878, 1995
3. Makino H, Shikata K, Hironaka K, Kushiro M, Yamasaki Y, Sugimoto H, Ota Z, Araki N, Horiuchi S: *Kidney Int* 48: 517 –526,1995
4. Suzuki D, Yagame M, Jinde K, Naka R, Yano N, Endoh M, Kaneshige H, Nomoto Y, Sakai H: J *Diabetes Complications* 10: 314 –319,1996
5. Imai N, Nishi S, Suzuki Y, Karasawa R, Ueno M, Shimada H, Kawashima S, Nakamaru T, Miyakawa Y, Araki N, Horiuchi S, Gejyo F, Arakawa M: *Nephron* 76: 153 –160,1997
6. Murata T, Nagai R, Ishibashi T, Inomuta H, Ikeda K, Horiuchi S: *Diabetologia* 40: 764 –769,1997
7. Kume S, Takeya M, Mori T, Araki N, Suzuki H, Horiuchi S, Kodama T, Miyauchi Y, Takahashi K: *Am J Pathol.* 147: 654 –667, 1995
8. Sakata N, Imanaga Y, Meng J, Tachikawa Y, Takebayashi S, Nagai R, Horiuchi S, Itabe H, Takano T: *Atherosclerosis* 141: 61 –75,1998
9. Sakata N, Imanaga Y, Meng J, Tachikawa Y, Takebayashi S, Nagai R, Horiuchi S: *Atherosclerosis* 142: 67 –77,1999
10. Kislinger T, Fu C, Huber B, Qu W, Taguichi A, Du Yan S, Hofmann M, Yan SF, Pischetsrieder M, Stern D, Schmidt AM: *J Biol Chem* 274: 31740 –31749,1999

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