Datasheet for ABIN2345148

Carboxy Methyl Lysine ELISA Kit

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Overview

Quantity:	96 tests
Target:	Carboxy Methyl Lysine (CML)
Reactivity:	Chemical
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Brand:	OxiSelect™
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Sensitivity:	50 ng/mL
Components:	1. 96-well Protein Binding Plate : One strip well 96-well plate. 2. Anti-CML Antibody (1000X) : One 10 µL vial of anti-CML antibody. 3. Secondary Antibody, HRP Conjugate (1000X) : One 20 µL vial. 4. Assay Diluent : One 50 mL bottle. 5. 10X Wash Buffer : One 100 mL bottle. 6. Substrate Solution : One 12 mL amber bottle. 7. Stop Solution (Part. No. 310808): One 12 mL bottle. Box 2 (shipped on blue ice packs)
Material not included:	1. Protein samples such as purified protein, plasma, serum, cell lysate 2. 1X PBS

Product Details

3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Target Details

Target:	Carboxy Methyl Lysine (CML)
Alternative Name:	Nepsilon-(Carboxymethyl) Lysine (CML) (CML Products)
Target Type:	Amino Acid
Background:	The non-enzymatic reaction of reducing carbohydrates with lysine side chains and N-terminal amino groups of macromolecules (proteins, phospholipids and nucleic acids) is called the Maillard reaction or glycation. The products of this process, termed advanced glycation end products (AGEs), adversely affect the functional properties of proteins, lipids and DNA. Tissue levels of AGE increase with age and the formation of AGEs is predominantly endogenous, though these products can also be derived from exogenous sources such as food and tobacco smoke. AGE modification of proteins can contribute to the pathophysiology of aging and long-term complications of diabetes, atherosclerosis and renal failure. AGEs also interact with a variety of cell-surface AGE-binding receptors (RAGE), leading either to their endocytosis and degradation or to cellular activation and pro-oxidant or pro- inflammatory events. Although several AGE structures have been reported, it was demonstrated that Nε-(carboxymethyl) lysine (CML) is a major antigenic AGE structure. CML concentration is increased in patients who have diabetes with complications, including nephropathy, retinopathy, and atherosclerosis. CML is also recognized by receptor for AGE (RAGE), and CML-RAGE interaction activates cell signaling pathways such as NF-κB. The quantity of CML adduct in protein samples is determined by comparing its absorbance with that of a known CML-BSA standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown protein samples.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Detect CML as low as 50 ng/mL from a variety of samples • CML-modified BSA included as standard • Compatible with cell lysates, serum, plasma, purified proteins, and other protein-containing samples

Application Details

Plate:	Uncoated
Protocol:	First, a CML conjugate is coated on the ELISA plate. The unknown CML protein samples or CML- BSA standards are then added to the CML conjugate preabsorbed plate. After a brief incubation, the anti-CML monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The content of CML protein adducts in unknown samples is determined by comparison with the predetermined CML-BSA standard curve.
Reagent Preparation:	• CML Conjugate Coated Plate: Note: The CML Conjugate coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately. 1. Immediately before use, prepare 1X Conjugate Diluent by diluting the 100X Conjugate Diluent in 1X PBS. Example: Add 50 µL to 4.95 mL of 1X PBS. 2. Immediately before use, prepare 1X CML Conjugate by diluting the 1000X CML Conjugate in 1X Conjugate Diluent. Example: Add 5 µL of 1000X CML Conjugate to 4.995 mL of 1X Conjugate Diluent. 3. Add 100 µL of the 1X CML Conjugate to each well to be tested and incubate overnight at 4 °C. Remove the CML Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hr at room temperature on an orbital shaker. Transfer the plate to 4 °C and remove the Assay Diluent immediately before use. • 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity. • Anti-CML Antibody and Secondary Antibody: Immediately before use, dilute the Anti-CML antibody 1:1000 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.
Assay Procedure:	1. Prepare and mix all reagents thoroughly before use. Each CML sample including unknown and standard should be assayed in duplicate. 2. Add 50 µL of unknown sample or CML-BSA standard to the wells of the CML Conjugate coated plate. If needed, unknown samples may be diluted in 1X PBS containing 0.1 % BSA before adding. Incubate at room temperature for 10 minutes on an orbital shaker. 3. Add 50 µL of the diluted anti-CML antibody to each well, incubate at room temperature for 1 hour on an orbital shaker. 4. Wash 3 times with 250 µL of 1X Wash Buffer with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer. 5. Add 100 µL of the diluted Secondary Antibody-HRP Conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 3 times according to step 4 above. 6. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well. Incubate at room temperature for 2-20 minutes on an orbital shaker. Note: Watch plate carefully, if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation. 7. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be

Application Details

	read immediately (color will fade over time). 8. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length. 5
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C/-20 °C
Storage Comment:	Aliquot and store the Anti-CML Antibody, CML-BSA Standard, CML Conjugate and 100X Conjugate Diluent at -20°C to avoid multiple freeze/thaw cycles. Store all other components at 4°C. 3

Publications

Product cited in:

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Oh, Kang, Ooi, Choi, Sage, Rhee: "Overexpression of SPARC in human trabecular meshwork increases intraocular pressure and alters extracellular matrix." in: **Investigative ophthalmology & visual science**, Vol. 54, Issue 5, pp. 3309-19, (2013) (PubMed).

Muruganandan, Parlee, Rourke, Ernst, Goralski, Sinal: "Chemerin, a novel peroxisome proliferator-activated receptor gamma (PPARgamma) target gene that promotes mesenchymal stem cell adipogenesis." in: **The Journal of biological chemistry**, Vol. 286, Issue 27, pp. 23982-95, (2011) (PubMed).

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