Product Manual

OxiSelect™ N^{ε}-(carboxymethyl) lysine (CML) Immunoblot Kit

Catalog Number

STA-313 **10 blots**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Creating Solutions for Life Science Research

Introduction

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 N^{ε}-(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.

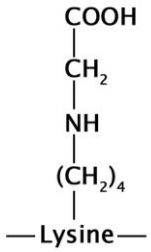

Figure 1. Structure of N^{ε}-(carboxymethyl) lysine (CML)

The OxiSelect™ N^{ε}-(carboxymethyl) lysine (CML) Immunoblot Kit is a simple and complete system for the detection of CML-protein adducts. The kit includes antibodies for the detection of CML in samples. The kit also includes a CML-BSA Immunoblot Control as a positive control. Each kit provides sufficient quantities to perform at least 10 blots (7.5 cm x 8.5 cm).

Related Products

1. STA-314: OxiSelect™ CML-BSA Control
2. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
3. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)
4. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
5. STA-337: OxiSelect™ 8-iso-Prostaglandin F2a Activity Assay Kit
6. STA-344: OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit
7. STA-342: OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence)
8. STA-347: OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)

9. STA-816: OxiSelect™ N-epsilon-(Carboxymethyl) Lysine (CML) Competitive ELISA Kit
10. STA-817: OxiSelect™ Advanced Glycation End Products (AGE) Competitive ELISA Kit
11. STA-832: OxiSelect™ MDA Competitive ELISA Kit
12. STA-838: OxiSelect™ HNE Adduct Competitive ELISA Kit

Kit Components

1. Rabbit Anti-CML Antibody (Part No. 231301): One tube – 100 µL.
2. Secondary Antibody, HRP-conjugate (Part No. 230805): One tube – 100 µL.
3. CML-BSA Immunoblot Control (Part No. 231302): One tube – 100 µL of 0.1 µg/mL of CML-BSA adduct (ready-to-use in 1X SDS-PAGE reducing sample buffer, pre-boiled).

Materials Not Supplied

1. Protein molecular weight standards
2. Polyacrylamide gels such as precast gels
3. Electrophoresis buffers
4. Reducing SDS Sample Buffer
5. Electrophoresis and western blot transfer systems
6. Immunoblotting buffers such as TBST (20 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.05% Tween-20)
7. PVDF or nitrocellulose membrane
8. Methanol
9. Non-fat dry milk
10. ECL reagents

Storage

Store all components at -20ºC. If the entire kit will not be used at once, aliquot kit components to avoid multiple freeze/thaw cycles.

Assay Protocol

I. Electrophoresis and Transblotting

1. Prepare samples for electrophoresis with reducing SDS Sample Buffer.
2. Load 10 µL of CML-BSA Immunoblot Control (provided pre-boiled and ready-to-use) or prepared samples to wells of a polyacrylamide gel. It is recommended to include a pre-stained MW standard (as indicator of a successful transfer in step 3). Run the gel as per the manufacturer's instructions.
3. Transfer the gel proteins to a nitrocellulose or PVDF membrane as per the manufacturer's instructions.

II. Immunoblotting

1. After the transfer, remove the blot and wash once in TBST for 5 minutes.

2. Block the membrane with 5% non-fat dry milk in TBST for 1 hr at room temperature with constant agitation (blocking can also be performed overnight at 4°C).
3. Wash the blocked membrane three times with TBST, 5 minutes for each wash.
4. Incubate the membrane with Rabbit Anti-CML Antibody, freshly diluted 1:1000 in 5% non-fat dry milk/TBST, for 1-2 hr at room temperature with constant agitation (incubation can also be performed overnight at 4°C).
5. Wash the blotted membrane three times with TBST, 5 minutes for each wash.
6. Incubate the membrane with Secondary Antibody, HRP-conjugate, freshly diluted 1:1000 in 5% non-fat dry milk/TBST, for 1 hr at room temperature with constant agitation.
7. Wash the blotted membrane five times with TBST, 5 minutes for each wash.
8. Use the detection method of your choice. We recommend enhanced chemiluminescence reagents from Pierce.

Example of Results

The following figure demonstrates typical blot results for the CML-BSA Immunoblot Control. One should use the data below for reference only. This data should not be used to interpret actual results.

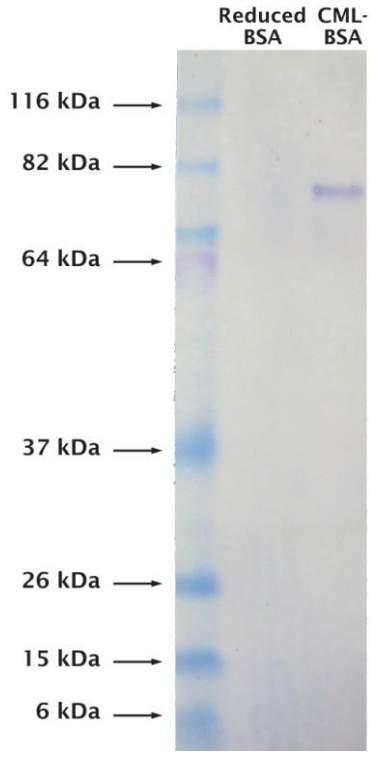

Figure 2: Immunoblotting of CML-BSA Control. CML-BSA Immunoblot Control, was first electroblotted onto nitrocellulose membrane. CML was detected by immunoblotting with anti-CML antibody as described in the Assay Protocol.

References

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Recent product citation

Abdallah, J. et al. (2016). The DJ-1 superfamily members YhbO and YajL from Escherichia coli repair proteins from glycation by methylglyoxal and glyoxal. *Biochem Biophys Res Commun*. doi:10.1016/j.bbrc.2016.01.068.

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