Product Manual

# OxiSelect™ Protein Carbamylation Sandwich ELISA Kit

**Catalog Number**

**STA- 877** **96 assays**

**FOR RESEARCH USE ONLY**
**Not for use in diagnostic procedures**


## Introduction

Carbamylation is a post-translational modification which occurs throughout the lifespan of proteins in vivo. Carbamylation results from the binding of isocyanic acid, spontaneously derived from high concentrations of urea and leading to the formation of carbamyl-lysine (CBL) (Figure 1). The carbamylation of proteins is usually associated with a partial or complete loss of protein function. It is known that elevated urea directly induces the formation of potentially atherogenic carbamylated LDL (cLDL). High blood concentrations of urea leading to the carbamylation process were detected in uremic patients and patients with end-stage renal disease.

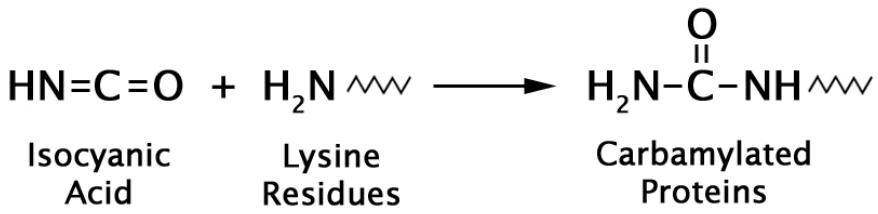


**Figure 1: Formation of Carbamyl-Lysine (CBL) During Carbamylation of Proteins.**

The OxiSelect™ Protein Carbamylation Sandwich ELISA Kit is an enzyme immunoassay developed for rapid detection and quantitation of protein carbamylation. The quantity of carbamylated adduct in protein samples is determined by comparing its absorbance with that of a known CBL-BSA standard curve. The kit has a detection sensitivity limit of 1.5 ng/mL of CBL-BSA. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown protein samples.

## Assay Principle

CBL-BSA standards or protein samples are added to an Anti-CBL Antibody Coated Plate for 2 hours at 37ºC. The CBL protein adducts present in the sample or standard are probed with an anti-CBL antibody, followed by an HRP conjugated secondary antibody. The protein CBL adduct content in an unknown sample is determined by comparing against a standard curve prepared from CBL-BSA standards.

## Related Products

1. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
2. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
3. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
4. STA-811: OxiSelect™ Methylglyoxal (MG) Competitive ELISA Kit
5. STA-816: OxiSelect™ $N^{\varepsilon}$-(carboxymethyl) lysine (CML) Competitive ELISA Kit
6. STA-817: OxiSelect™ Advanced Glycation End Products (AGE) Competitive ELISA Kit
7. STA-832: OxiSelect™ MDA Adduct Competitive ELISA Kit
8. STA-838: OxiSelect™ HNE-His Adduct Competitive ELISA Kit

## Kit Components

**Box 1 (shipped at room temperature)**

1. 96-Well Anti-CBL Antibody Coated Plate (Part No. 287701): One strip well 96-well plate.
2. Anti-CBL Antibody (1000X) (Part No. 287702): One 15 µL vial of anti-CBL antibody.
3. Secondary Antibody, HRP Conjugate (Part No. 231009): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

**Box 2 (shipped on blue ice packs)**

1. CBL-BSA Standard (Part No. 287703): One 40 µL vial of 10 µg/mL CBL-BSA in Assay Diluent.

## Materials Not Supplied

1. Protein samples such as purified protein, plasma, serum, cell lysate
2. 1X PBS
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)

## Storage

Upon receipt, aliquot and store the CBL-BSA Standard at -20ºC to avoid multiple freeze/thaw cycles. Store all other kit components at 4ºC.

## Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Anti-CBL Antibody and Secondary Antibody: Immediately before use dilute the Anti-CBL antibody 1:1000 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

## Preparation of Standard Curve

Prepare a series of CBL-BSA standards according to Table 1.

| Standard Tubes | 10 µg/mL CBL-BSA (µL) | Assay Diluent (µL) | CBL-BSA (ng/mL) |
|---|---|---|---|
| 1 | 8 | 792 | 100 |
| 2 | 400 of tube #1 | 400 | 50 |
| 3 | 400 of tube #2 | 400 | 25 |
| 4 | 400 of tube #3 | 400 | 12.5 |
| 5 | 400 of tube #4 | 400 | 6.25 |
| 6 | 400 of tube #5 | 400 | 3.125 |
| 7 | 400 of tube #6 | 400 | 1.5625 |
| 8 | 0 | 400 | 0 |

**Table 1. Preparation of CBL-BSA Standard Curve**

## Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample including unknown and CBL-BSA standard should be assayed in duplicate.
2. Add 100 µL of unknown sample or CBL-BSA standard to the Anti-CBL Antibody Coated Plate. Incubate at 37ºC for at least 2 hours or 4ºC overnight.
3. 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.
4. Add 100 µL of the diluted anti-CBL antibody to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 3 times according to step 3 above.
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. Warm Substrate Solution to room temperature during this incubation.
6. Wash the strip wells 3 times according to step 3 above.
7. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   *Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.*
8. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).
9. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

## Example of Results

The following figures demonstrate typical Protein Carbamylation Sandwich ELISA Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

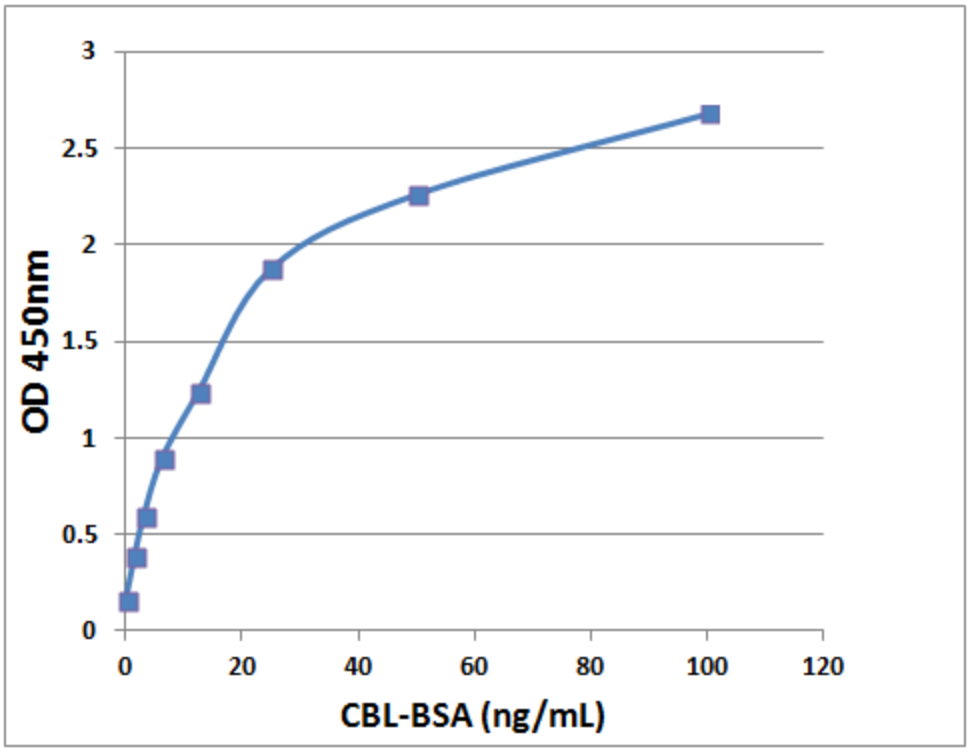


**Figure 2: Protein Carbamylation Sandwich ELISA Standard Curve.**

## References


1. Wang, Z., et al. (2007). *Nat. Med*. **13**:1176-1184.
2. Kraus, L.M., et al. (2001). *Swiss Med. Wkly*. **131**:139-144
3. Sirpal, S. (2009). *Clin. Sci. (Lond.)* **116**:681-695.
4. Balion, C.M., et al. (1998). *Kidney Int.* **53**:488-495.
5. Asci, G., et al. (2008). *Nephrology (Carlton)* **13**:480-486.
6. Jaisson, S., et al. (2007). *FEBS Lett.* **581**:1509-1513.
7. Garnotel, R., et al. (2004). *FEBS Lett.* **563**:13-16.


## Recent Product Citations


1. Bright, R. et al. (2017). The effect of triclosan on posttranslational modification of proteins through citrullination and carbamylation. *Clin. Oral Investig.* doi:10.1007/s00784-017-2137-8.
2. Tajerian, M. et al. (2016). Identification of KRT16 as a target of an autoantibody response in complex regional pain syndrome. *Exp, Neurol.* **287**:14-20.

3. Sun, J. T. et al. (2016). Increased carbamylation level of HDL in end-stage renal disease: Carbamylated-HDL attenuated endothelial cell function. *Am J Physiol Renal Physiol.* doi:10.1152/ajprenal.00508.2015.

## Warranty

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