KAMIYA BIOMEDICAL COMPANY

Protein Carbamylation Sandwich ELISA

For rapid detection and quantitation of protein carbamylation

Cat. No. KT-973

For Research Use Only. Not for use in diagnostic procedures.

Product Information

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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 samples and samples with end-stage renal disease.

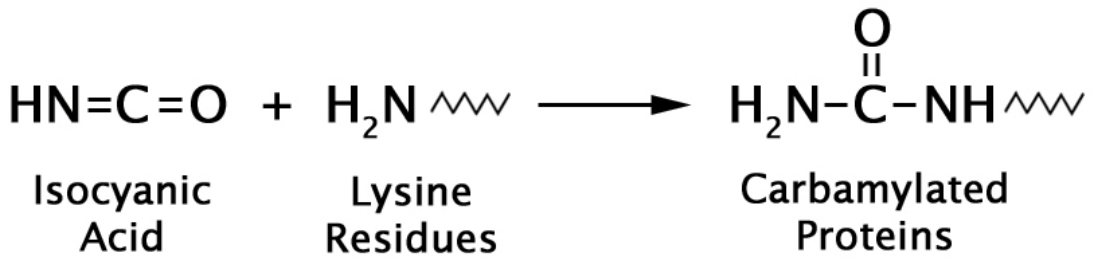

Figure 1: Formation of Carbamyl-Lysine (CBL) During Carbamylation of Proteins.

The 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 calibration 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 calibration curve and unknown protein samples.

PRINCIPLE

CBL-BSA calibrators 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 calibrator 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 calibration curve prepared from CBL-BSA calibrators.

COMPONENTS

1. 96-Well Anti-CBL Antibody Coated Plate: One strip well 96-well plate.
2. Anti-CBL Antibody (1,000X): One 15 µL vial of anti-CBL antibody.
3. Secondary Antibody, HRP Conjugate: 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: One 12 mL bottle.
8. CBL-BSA Calibrator: One 40 µL vial of 10 µg/mL CBL-BSA in Assay Diluent.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Protein samples such as purified protein, plasma, serum, cell lysate
2. 1X PBS
3. 10 µL to 1,000 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 Calibrator at -20ºC to avoid multiple freeze/thaw cycles. Store all other kit components at 4ºC.

REAGENT PREPARATION

• 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 1:1,000 and Secondary Antibody 1:1,000 with Assay Diluent. Do not store diluted solutions.

CALIBRATION CURVE PREPARATION

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

Calibrator 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 Calibration Curve

ASSAY PROCEDURE

1. Prepare and mix all reagents thoroughly before use. Each sample including unknown and CBL-BSA calibrator should be assayed in duplicate.

2. Add 100 µL of unknown sample or CBL-BSA calibrator 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.

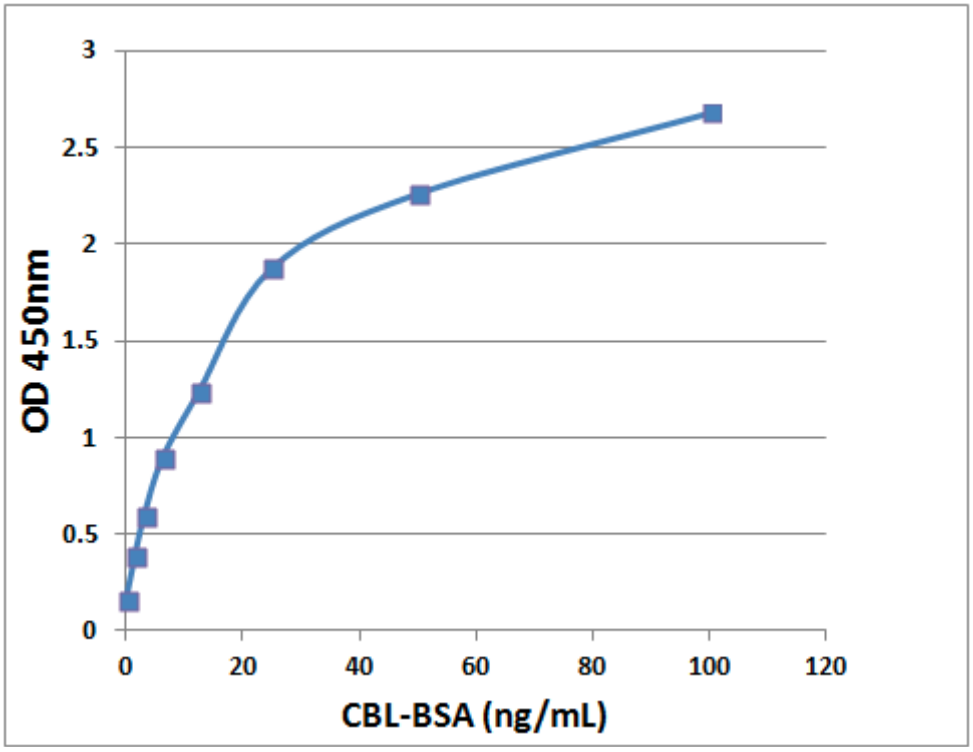

Figure 2: Protein Carbamylation Sandwich ELISA Calibration Curve.

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