Protein Carbamylation ELISA Kit

Enzyme Immunoassay for the quantification of protein carbamylation in purified protein, plasma, serum, and cell lysate samples.

Catalog number: ARG81220

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INTRODUCTION

Carbamylation (carbamoylation) is a post-translational modification resulting from the nonenzymatic reaction between isocyanic acid and free functional groups of proteins, in particular with the free amino groups. This reaction alters structural and functional properties of proteins and results in faster aging of proteins. Urea present in the body can be transformed into cyanate and its more reactive form, isocyanic acid. High concentration of urea is associated with some diseases, especially with chronic renal failure and atherosclerosis. In human tissues, urea and cyanate are in equilibrium in aqueous solutions. Surprisingly, concentration of isocyanate in the body is much lower than it would appear from the kinetic parameters of urea decomposition. The low concentration of isocyanic acid results from its high reactivity and short half-life. The carbamylation of proteins may have a negative impact on their biological activity and may contribute to the deterioration of patients with chronic renal failure. [Provide by *Postepy Hig Med Dosw*: Carbamylation of proteins – mechanism, causes and consequences]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique.

A capture antibody specific for carbamyl-lysine (CBL) has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any CBL present is bound by the immobilized antibody. After washing away any unbound substances, an antibody-conjugate specific for CBL is added to each well and incubate. After washing away any unbound antibody, an HRP conjugate antibody added to the wells. After washing away any unbound substances, the substrate solution (TMB substrate) is added to the wells and color develops in proportion to the amount of CBL bound in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm.The concentration of CBL in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, aliquot and store the CBL-BSA Standard at -20°C to avoid multiple freeze/thaw cycles. Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
Standards (10 µg/ml CBL-BSA)	40 µL	–20°C.
Diluent Buffer	50 ml (ready to use)	4°C
Antibody Conjugate (Anti-CBL antibody)	15 µL	4°C
HRP Conjugate Antibody.	20 µL	4°C
10X Washing Buffer	100 ml	4°C
TMB substrate	12 ml (ready to use)	4°C (protect from light)
STOP solution	12 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Tissue/Feces Homogenizer (optional)
- 1X PBS
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- The Stock Standard should be aliquoted into smaller portions before use to ensure product integrity and store the aliquoted Stock Standard at -20°C. Avoid repeated freeze-thaw cycles
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB

solution to glass, foil or metal. If the solution is blue before use, do NOT use it.

- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Use a new adhesive plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Collect the plasma layer and store on ice.

Cells lysate: Homogenize 50-200 mg of the cell pellet in 0.5-2 mL of ice cold PBS using a mortar and pestle or by dounce homogenization. Incubate the homogenate at 4°C for 20 minutes. Transfer the homogenate to a centrifuge tube and centrifuge at 12000 x g for 20 minutes. Recover the supernatant and transfer to a fresh tube. Store resuspended sample at -20°C or colder until ready to test by ELISA.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum/plasma sample.
3. Aliquot samples for testing and store at -80°C. Avoid repeated freeze-thaw cycles. Perform dilutions in Diluent buffer as necessary.
4. Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **1X Wash Buffer**: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (E.g., add 50 ml of 10X Wash buffer into 450 ml of distilled water to a final volume of 500 ml) The 1X Washing Buffer is stable for up to 4 weeks at 2-8°C.
- **Antibody conjugate and HRP conjugate antibody**: Immediately before use dilute the Antibody conjugate Anti-CBL antibody 1:1000 and the HRP Conjugate antibody 1:1000 with Diluent buffer. Do not store diluted solutions.
- **Standards (CBL-BSA):** Prepare a series dilution of CBL-BSA standards with Diluent buffer. The Diluent buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted with Diluent buffer as according to the suggested concentration table below:

Note: Dilutions for the standard must be made and applied to the plate immediately. S0 serves as background.

Standard tubes	CBL-BSA (ng/mL)	Diluent buffer (µL)	CBL-BSA standard (µL)
S1	100	792	8 of 10 µg/ml stock standard
S2	50	400	400 of S1
S3	25	400	400 of S2
S4	12.5	400	400 of S3
S5	6.25	400	400 of S4
S6	3.13	400	400 of S5
S7	1.56	400	400 of S6
S0	0	400	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls should be assayed in duplicates.

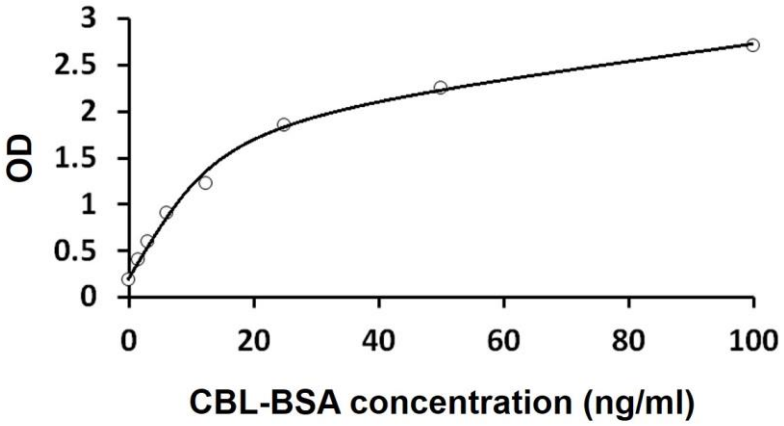
1. Add **100 µL** of **sample or standard** to the Antibody-coated microplate.
2. Incubate at **37ºC** for at least **2 hours** or **4ºC overnight**.
3. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of the diluted **Antibody Conjugate** to each well. Incubate at **RT** for **1 hour** on a microplate shaker.
5. Aspirate each well and **wash as step 3**.
6. Add **100 µL** of the diluted **HRP Conjugate** to each well. Incubate at **RT** for **1 hour** on a microplate shaker.
7. Aspirate each well and **wash as step 3**.
8. Add **100 µl** of **TMB Substrate** to each well, including the blank wells. Incubate for **2-30 minutes** at room temperature in the dark.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Add **100 µl** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at **450nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the

absorbance within 30 minutes after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD CURVE

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



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Intra-assay and Inter-assay precision

The CV value of intra-assay and inter-assay precision was ≤ 10%.

Sensitivity

1.5 ng/ml