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ab238540 N-epsilon-(Carboxyethyl) Lysine (CEL) Assay Kit

For the quantitative rapid detection and quantitation of CEL protein adducts in samples such as purified protein, plasma, serum and cell lysates.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

N-epsilon-(Carboxyethyl) Lysine (CEL) Assay Kit (ab238540) is designed for the rapid detection and quantitation of CEL protein adducts.

First, a CEL conjugate is coated on the ELISA plate. The unknown CEL protein samples or CEL-BSA standards are then added to the CEL conjugate preabsorbed plate. After a brief incubation the anti-CEL monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The content of CEL protein adducts in unknown samples is determined by comparison with the pre-determined CEL-BSA standard curve. Despite the structure similarity between CEL and CML, the anti-CEL specific antibody in this kit will not cross react with CML protein adducts.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed.

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Add 50 µL standard or sample to wells of CEL Conjugate coated plate and incubate for 10 minutes at room temperature.

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Add 50 µL of the diluted anti-CEL antibody and incubate for 1 hour at room temperature.

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Washing steps with 250 µL 1X Wash Buffer.

⇩

Add 100 µL diluted Secondary Antibody-HRP Conjugate per well and incubate for 1 h. Wash as before with 1X Wash buffer.

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Add 100 µL of warm Substrate Solution and incubate for 5-20 minutes at room temperature.

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Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Read absorbance immediately on a microplate reader using 450 nm.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at +4°C immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage condition
96-Well Protein Binding Plate	1 unit	+4°C
Anti-CEL Antibody (1000X)	10 µL	-20°C
Secondary Antibody, HRP Conjugate (1000X)	20 µL	+4°C
Assay Diluent	50 mL	+4°C
10X Wash Buffer	100 mL	+4°C
Substrate Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
CEL-BSA Standard	30 µL	-20°C
CEL Conjugate	20 µL	-20°C
100X Conjugate Diluent	300 µL	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at O.D. 450 nm (620 nm as optional reference wave length).

6. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.
- Any components not listed here are ready to use as supplied.

6.1 CEL Conjugate Coated Plate

Δ Note: The CEL 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.

6.1.1 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.

6.1.2 Immediately before use, prepare 1.0 µg/mL of CEL Conjugate by diluting the 1.0 mg/mL CEL Conjugate in 1X Conjugate Diluent. Example: Add 5 µL of 1.0 mg/mL CEL Conjugate to 4.995 mL of 1X Conjugate Diluent and mix well.

6.1.3 Add 100 µL of **1 µg/mL** CEL Conjugate to each well to be tested and incubate overnight at 4°C.

6.1.4 Remove the CEL Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid.

6.1.5 Add 200 µL of Assay Diluent to each well and block for 1 h at room temperature on an orbital shaker. Transfer the plate to 4°C and remove the Assay Diluent immediately before use.

6.2 1X Wash Buffer:

6.2.1 Dilute the 10X Wash Buffer to 1X with deionized water.

6.2.2 Stir to homogeneity.

6.3 Anti-CEL Antibody and Secondary Antibody

6.3.1 Immediately before use dilute the Anti-CEL antibody 1:1000 and Secondary Antibody 1:1000 with Assay Diluent.

6.3.2 Do not store diluted solutions.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Prepare a dilution series of CEL-BSA standards in the concentration range of 0 to 25 µg/mL by diluting the 1 mg/mL CEL-BSA Standard in Assay Diluent as per the table below.

Standard #	1 mg/mL CEL-BSA Standard (µL)	Assay Diluent (µL)	CEL -BSA (µg/mL)
1	10	390	25
2	200 of standard #1	200	12.5
3	200 of standard #2	200	6.25
4	200 of standard #3	200	3.13
5	200 of standard #4	200	1.56
6	200 of standard #5	200	0.78
7	200 of standard #6	200	0.39
8	200 of standard #7	200	0.20
9	200 of standard #8	200	0.10
10	0	200	0

8. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.

9. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

Δ Note: If testing mouse or rat plasma or serum, the IgG must be completely removed from each sample prior to testing, such as with Protein A or G beads. Additionally, a control well without primary antibody should be run for each sample to determine background signal.

9.1 Add 50 µL of unknown sample or CEL-BSA standard to the wells of the CEL 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.

9.2 Add 50 µL of the diluted anti-CEL antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.

9.3 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.

9.4 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 9.3 above.

9.5 Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well. Incubate at room temperature for 5-20 minutes on an orbital shaker.

Δ Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9.6 Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).

9.7 Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

10. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

10.1 Average the duplicate reading for each standard, control and sample.

10.2 Subtract the mean value of the blank (Standard #10) from all standards, controls and sample readings. This is the corrected absorbance.

10.3 If significant, subtract the sample background control from sample readings.

10.4 Plot the corrected values for each standard as a function of the final concentration of CEL Adduct.

10.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

10.6 Apply the corrected sample OD reading to the standard curve to get CEL Adduct amount in the sample wells.

10.7 Concentration of CEL Adduct in [B units / V units] in the test samples is calculated as:

$$CEL\ Adduct\ concentration = \frac{B}{V} * D$$

Where:

B = amount of CEL Adduct in the sample well calculated from standard curve in µg/mL

V = sample volume added in the sample wells in µL

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

11. Typical Data

Typical standard curve - data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

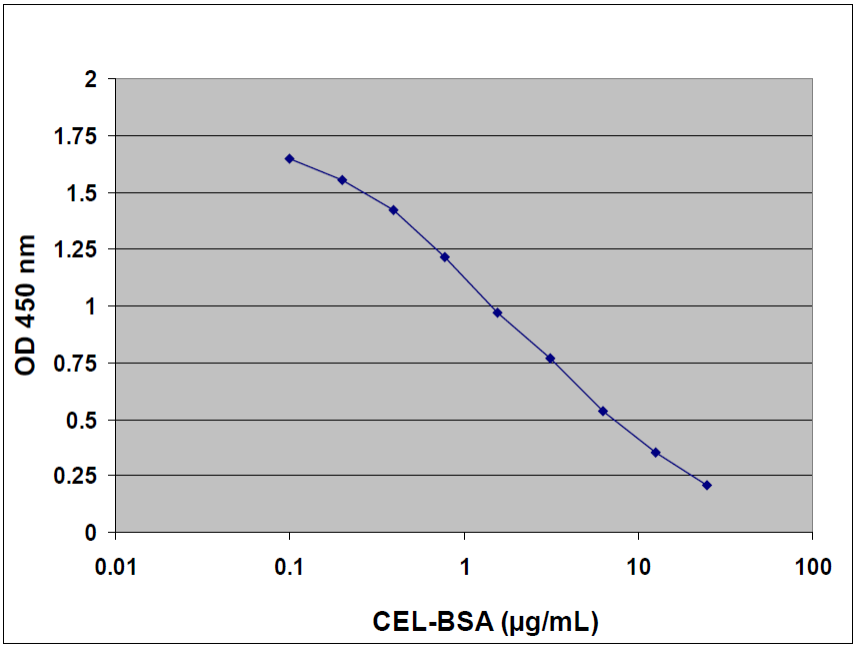

Figure 1. Typical Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

12. Species Reactivity

This kit is not species specific and can be used with samples from any species.

Please contact our Technical Support team for more information.

13. Notes

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