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ab238536
Protein Carbonyl ELISA Kit

For the quantitative measurement of protein carbonyls in plasma, serum, cell and tissue lysates or purified proteins.

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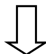
1. Overview

Protein Carbonyl ELISA Kit (ab238536) is designed for the quantitative measurement of protein carbonyls in plasma, serum, cell and tissue lysates or purified proteins.

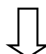
With this kit protein samples are first allowed to adsorb to wells of a 96-well plate and then react with dinitrophenylhydrazine (DNPH), which allows for derivatization of the carbonyl group. There are no concentration or precipitation steps, that contribute to sample loss. The kit allows detection of as little as 10 µg/mL using a standard microplate reader. The quantity of protein carbonyls in protein sample is determined by comparing its absorbance with that of a known reduced/oxidized BSA standard curve.

2. Protocol Summary

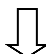
Prepare all reagents, samples, and standards as instructed



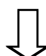
Add 100 µL standard or sample to appropriate wells. Cover and incubate for at least 2 hours at 37°C or 4°C overnight. Wash plate 3 times with 250 µL 1X PBS.



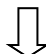
Add 100 µL DNPH Working Solution to each well. Cover and incubate for 45 minutes at room temperature in the dark. Washing steps with 250 µL PBS/Ethanol and then 250 µL 1X PBS.



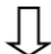
Add 200 µL Blocking solution per well and incubate the plate at room temperature for 1-2 hours. Wash with 250 µL 1X Wash buffer.



Add 100 µL diluted anti-DNP antibody and incubate for 1 hour at room temperature. Wash with 1X Wash buffer.



Add 100 µL HRP conjugated secondary antibody and incubate for 1 hour at room temperature. Wash with 1X Wash buffer.



Add 100 µL warm Substrate solution and incubate from 2 – 30 minutes, then add 100 µL of stop solution. Read absorbance of each well on a plate 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.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Materials Supplied, and Storage and Stability

- Store kit at +4°C immediately upon receipt and check below for storage for individual components.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity		Storage condition
	96 test	32 test	
Protein Binding Strip Well Plate	96 well	32 well	+4°C
Anti-DNP Antibody (1000X)	20 µL	5 µL	+4°C
Secondary Antibody, HRP Conjugate (1000X)	20 µL	20 µL	+4°C
25X DNPH Solution	500 µL	500 µL	+4°C
2X DNPH Diluent	15 mL	15 mL	+4°C
Blocking Reagent	20 g	20 g	+4°C
10X Wash Buffer	100 mL	30 mL	+4°C
Substrate Solution	12 mL	4 mL	+4°C
Stop Solution	12 mL	4 mL	+4°C
Reduced BSA Standard	200 µL	75 µL	-20°C
Oxidized BSA Standard	200 µL	75 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 1X Wash Buffer:

6.1.1 Dilute the 10X Wash Buffer Concentrate to 1X with deionized water.
6.1.2 Stir to homogeneity.

6.2 1X DNPH Diluent

6.2.1 Dilute the 2X DNPH Diluent to 1X with deionized water.
6.2.2 Mix well.

6.3 Blocking Solution

6.3.1 Weigh out 5 g of Blocking Reagent.
6.3.2 Dissolve in 100 mL of 1X PBS, and store at 4°C for up to one week.

6.4 DNPH Working Solution

6.4.1 Based on the number of tests, freshly prepare appropriate amount of DNPH Working Solution by diluting the 25X DNPH Solution to 1X in 1X DNPH Diluent. For example: for 20 assays, transfer 80 µL of 25X DNPH Solution to a tube containing 1.92 mL of 1X DNPH Diluent, mix well and use it immediately.

6.5 Anti-DNP Antibody and Secondary Antibody

6.5.1 Immediately before use dilute the Anti-DNPH antibody 1:1000 and Secondary Antibody 1:1000 with 1X Blocking Solution.
6.5.2 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 Freshly prepare 10 µg/mL of reduced or oxidized BSA by diluting the 1 mg/mL BSA standards in 1X PBS. For example; add 20 µL to 1.98 mL of 1X PBS.

7.2 Prepare a series of carbonyl BSA standards by mixing the oxidized BSA and reduced BSA in the proper ratios according to Table 1.

Standard #	10 µg/mL Oxidized BSA (µL)	10 µg/mL Reduced BSA (µL)	[Protein Carbonyl] (nmol/mg)
1	400	0	7.5
2	320	80	6.0
3	240	160	4.5
4	160	240	3.0
5	80	320	1.5
6	40	360	0.75
7	20	380	0.375
8 (Blank)	0	400	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.

8.1 Perform a protein assay such as Bradford or BCA on all samples to determine the protein concentration.

Δ **Note:** Lysates should not be prepared in lysis buffer containing Triton X-100, NP-40, or Igepal CA-630 because these detergents interfere with protein coating of the plate unless the detergent concentration in the 10 µg/mL protein samples is no more than 0.001%. We recommend lysis by homogenization or sonication.

Δ **Note:** A high concentration of nucleic acid in cell or tissue lysates can erroneously contribute to higher estimation of carbonyl content. To remove nucleic acid, we recommend one of the following procedures:

1. Pretreat lysate with nuclease, followed by ammonium sulfate precipitation of high percentage saturation.

2. Add streptomycin sulfate or PEI to a final concentration of 1% and 0.5% respectively, incubate 30 minutes at room temperature and remove the nuclei acid precipitates by centrifuging at 6000 g for 10 minutes at 4°C.

8.2 Dilute each protein sample to 10 µg/mL in 1X PBS prior to use in the assay.

Δ **Note:** Samples with high concentrations of protein carbonyl content may be further diluted 5-10 fold in 10 µg/mL Reduced BSA. A titration may be performed to ensure the samples fall in the range of the standard curve.

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.

9.1 Prepare unknown samples according to the Sample Preparation section. Each 10 µg/mL protein sample and BSA Standard should be assayed in duplicate or triplicate.

9.2 Add 100 µL of 10 µg/mL protein samples or reduced/oxidized BSA standards to the 96-well Protein Binding Plate. Incubate at 37°C for at least 2 hours or 4°C overnight.

9.3 Wash wells 3 times with 250 µL 1X PBS per well. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess wash solution.

9.4 Add 100 µL of the DNPH Working Solution and incubate for 45 minutes at room temperature in the dark.

9.5 Wash wells with 250 µL of 1X PBS/Ethanol (1:1, v/v) with incubation on an orbital shaker for 5 minutes. Repeat washing a total of 5 times, aspirating between each. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess wash solution. Wash 2 times with 250 µL of 1X PBS.

9.6 Add 200 µL of Blocking Solution per well and incubate for 1-2 hours at room temperature on an orbital shaker.

9.7 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.

9.8 Add 100 µL of the diluted anti-DNP 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 9.7 above.

9.9 Add 100 µL of the diluted HRP conjugated secondary antibody to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 5 times according to step 9.7 above.

9.10 Warm Substrate Solution to room temperature. 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.

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

9.12 Read absorbance of each well on a plate reader using 450 nm as the primary wave length, using the fully reduced BSA standard as absorbance blank.

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 #8) 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 Protein Carbonyl.

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 protein carbonyl amount in the sample wells.

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

$$XX\ concentration = \frac{B}{V} * D$$

Where:

B = amount of protein carbonyl in the sample well calculated from standard curve in nmol/mg

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.

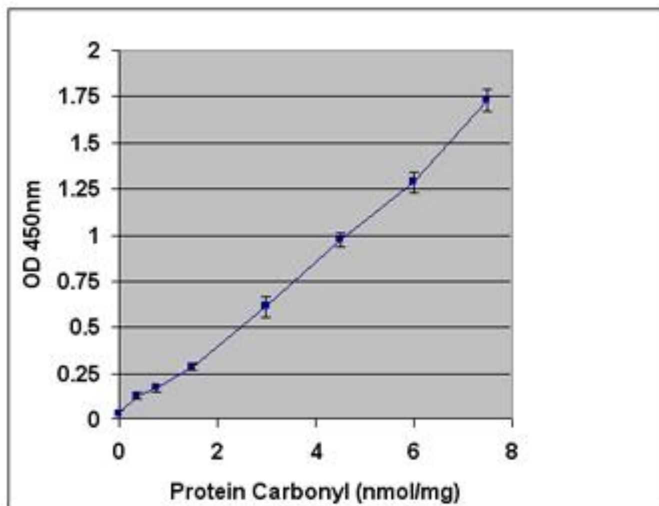


Figure 1. Typical Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

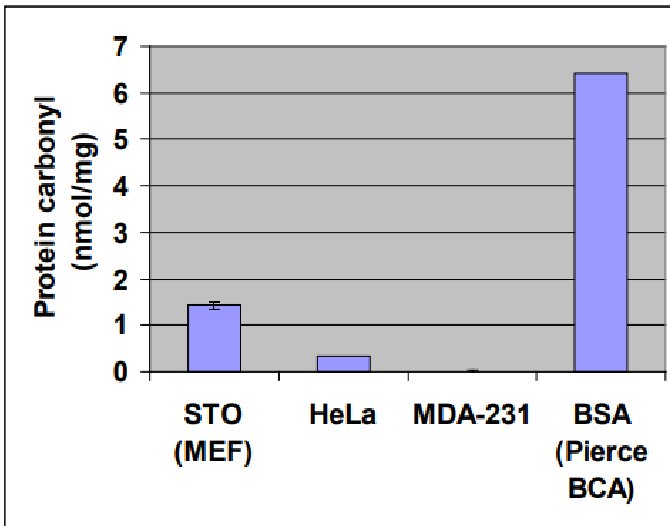


Figure 2. Amount of Protein Carbonyl Content for Cell Lysate and BSA Standard. STO (MEF), HeLa and MDA-231 cells were sonicated in 25mM HEPES, pH 7.5, 150 mM NaCl, 10 mM MgCl2, 1 mM EDTA, 2% Glycerol. Cell Lysates and BSA Standard from Pierce BCA Protein Assay were diluted to 10 µg/mL with 1X PBS and coated onto a 96-well Protein Binding Plate. The protein carbonyl levels were determined as described in the Assay Protocol.

12. Species Reactivity

The structure of protein carbonyl groups is the same regardless of species; therefore any protein sample from any species can be used with this assay.

Please contact our Technical Support team for more information.

13. Notes

Technical Support

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