



Product Manual

Protein Carbonyl ELISA kit

Manufactured by Enzo Life Sciences

Catalog # ALX-850-312-KI01

96-Well Enzyme-Linked Immunosorbent Assay Kit

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.



Product Manual

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Several of Enzo's products and product applications are covered by US and foreign patents and patents pending.



Reagents require separate storage conditions.



Check our website for additional protocols, technical notes and FAQs.



For proper performance, use the insert provided with each individual kit received.

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DESCRIPTION

The Protein Carbonyl Test Kit is an Enzyme-Linked Immunosorbent Assay (ELISA) for the measurement of protein carbonyls in biological samples. This kit contains materials for one 96 well plate. It will measure 27 samples as triplicates or 41 samples as duplicates if the whole plate is used at once.

The assay can be applied to biological fluids such as plasma, serum, bronchoalveolar lavage fluid and cerebrospinal fluid, to cell extracts and to other soluble protein samples.

For research use only.

INTRODUCTION

Protein carbonyls are formed by a variety of oxidative mechanisms and are sensitive indices of oxidative injury. The quantity of protein carbonyls in a protein sample can be determined by derivatising with dinitrophenylhydrazine(DNP) and measuring bound DNP colorimetrically or immunologically. The ELISA method enables carbonyls to be measured quantitatively with microgram quantities of protein.

ASSAY PRINCIPLE

A schematic diagram of the protein Carbonyl ELISA is shown in Figure 1. Samples containing protein are reacted with DNP; then the protein is non-specifically adsorbed to an ELISA plate. Unconjugated DNP and non-protein constituents are washed away. The adsorbed protein is probed with biotinylated anti-DNP antibody followed by streptavidin-linked horseradish peroxidase. Absorbances are related to a standard curve prepared for serum albumin containing increasing proportions of hypochlorous acid-oxidized protein that has been calibrated colorimetrically.

RECENT USE AND STABILITY

Reagents must be brought to room temperature prior to use.

It is not necessary to maintain PC kits under cool conditions for short term transportation. The kits are stable at room temperature for up to 6 weeks. If the kit is separated into freezer, fridge and bench as per instructions, it is stable until at least the expiry.

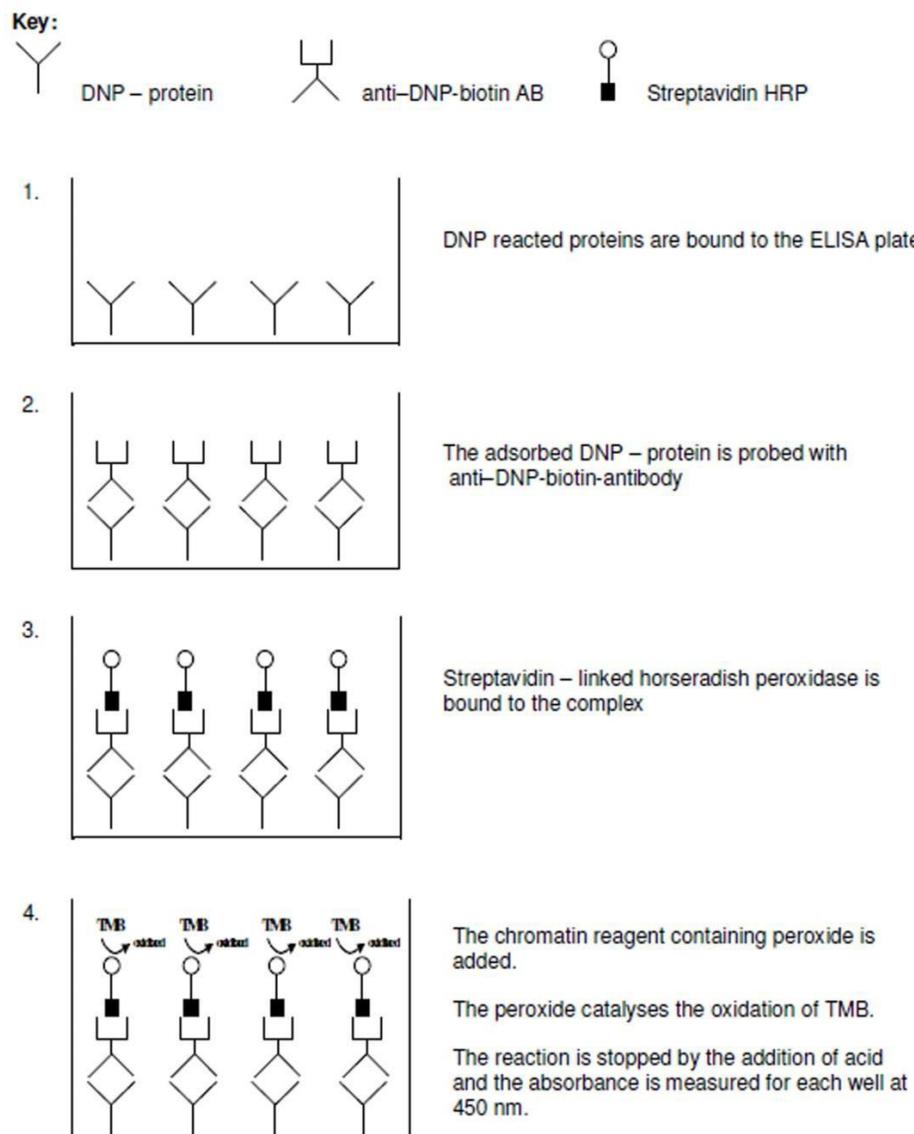
The stability of all other reagents is as shown in Notes 1-9 on page 4.

KIT COMPONENTS

The kit components (supplied materials) are sufficient for one plate. Each kit contains reagents and components labeled as follows:

- | | |
|-------------------------------------|--|
| A Instructions | G Anti-DNP biotin antibody |
| B ELISA 96-well plate + plate cover | H Streptavidin Horseradish Peroxidase |
| C ELISA buffer powder | I Chromatin reagent |
| D Blocking reagent | J Stopping reagent |
| E Dinitrophenylhydrazine | K Standards (6) |
| F Guanidine hydrochloride diluent | L Carbonyl control sample |
| | M Technical Bulletin indicating lot-specific standard concentrations |

Figure 1: Schematic diagram of the Protein Carbonyl ELISA



OTHER MATERIALS NEEDED



Bring Reagents to room temperature before using.

1. Single and multi-channel pipettes capable of delivering 5 μL , 10 μL , 100 μL , 200 μL , 250 μL and 1 mL.
2. Disposable pipette tips.
3. Containers for reagent mixing and pipetting reservoirs.
4. The use of an automated microwell washer is recommended.
5. Microwell spectrophotometric reader. The reader must read at **450 nm**.
6. Deionized water.
7. 1L and 100 mL measuring cylinders.
8. 1.5 mL and 0.5 mL (for low protein method) eppendorf tubes.
9. Trichloroacetic acid (TCA) 28% (w/v) for low protein method.

PRE-ASSAY PREPARATION

Note: *Gloves should be worn when handling reagents. Reagents G and H can disperse and attach to the sides of the microvial in transit and become difficult to accumulate into the bottom. Proceed with new instructions as below.*

1. ELISA Buffer(C).

Dissolve the contents of bottle (C) in one liter of deionized water. **Store at 4°C** for up to 4 weeks.

2. Blocking reagent (D).

Add 2 mL of ELISA Buffer to blocking reagent container (D). Mix well and transfer to a 100 mL measuring cylinder. Add ELISA Buffer to a final volume of 75 mL. Label this solution as “diluting blocking solution.” **Store at 4°C** for up to 4 weeks.

3. Dinitrophenylhydrazine (DNP) (E).

Add 1 mL of the DNP reagent (E) to 9 mL of guanidine hydrochloride (f). Label this solution as “Diluted DNP solution.” (**Please Note:** 10 mL of Diluted DNP solution is sufficient for 50 tests only. Increase the quantity if performing more than 50 tests, while keeping the ratio to

1 volume of E to 9 volumes of F). **Store at room temperature. If diluted reagent has been stored for more than 2 weeks, centrifuge at 8000g for 5 minutes and the use the supernatant for derivatization.**

4. **Anti-DNP biotin antibody (G). Store at -20°C until needed.**

Prepare this dilution directly before use. Add 0.5 mL of “diluted blocking solution” to the Anti-DNP biotin antibody microvial (G). Mix well and make up to 20 mL with “diluted blocking solution.”

5. **Streptavidin horseradish-peroxidase (HRP)(H). Store at 4°C until needed. Must NOT be frozen. Prepare this dilution directly before use.**

Add 0.5 mL of “diluted blocking solution” to the Streptavidin-HRP microvial (H). Mix well and make up to 20 mL with “diluted blocking solution.”

6. **Chromatin reagent (I). Store at 4°C until needed.**

Add 15 mL of Chromatin reagent (I) to 5 mL of ELISA buffer (C) and mix well.

Contamination of this solution may result in unintended color development. **Never pipette directly from container, and do not return unused reagent to main container.** Pour the required amount into a pipetting reservoir. 20 mL is needed.

7. **Stopping reagent (J).** (Ready-to-use.)
8. **Oxidized Protein Standards (K1-6). Store at -20°C until needed.**

Note:

- For this assay standard concentrations will be lot-specific. Please use the lot-specific values indicated in the technical bulletin provided to create the standard curve and perform sample calculations.
- **Standards (K1-6) and Control (L) can disperse and attach to the sides of the microvial in transit. Do a short spin/pulse in the centrifuge prior to opening these colored microvials. This is to ensure the powder is brought down to the base of the tube before the deionized water is added.**

Add **25 µL** of deionized water to each of the 6 oxidized protein samples, vortex, stand at 37°C for at least **2**

hours (or preferably overnight at room temperature), then re-vortex again to ensure ALL of the sample is dissolved. Reconstituted standards should be frozen at -80°C if another test is to be performed at a later date. Only use the top standard K6 (red/pink) if dealing with highly oxidized samples.

9. **Carbonyl control sample (L). Store at -20°C until needed.**

Add **25 µL** of deionized water to the carbonyl control sample, vortex, stand at 37°C for at least **2 hours** (or preferably overnight at room temperature), then revortex again to ensure ALL of the sample is dissolved. Reconstituted control should be frozen if another test is to be performed at a later date. The carbonyl levels should be close to the green standard (K4).



Bring Reagents to room temperature before using.

ASSAY PROCEDURE

1. Sample Derivatization with DNP

Note: Allow all reagents to equilibrate to room temperature (18-25°C) before performing the assay.

The assay is set up so that about 1 µg of derivatized protein is applied to each well of the ELISA plate. This is sufficient to saturate the well with protein, so some variation in the amount of protein applied to the plate will not affect the response. Two methods of sample preparation are described. The standard procedure is applicable to plasma or any sample with a protein concentration between 35 g/L and 80 g/L (*expected range for human plasma from healthy controls: 60-70 g/L*). The low protein method, which involves concentration of the protein by precipitation with trichloroacetic acid (TCA) should be used with more diluted samples.

Triplicate analyses of each sample are recommended (27 samples per whole plate).

A: **Standard procedure for samples containing 0.4 – 80 mg/mL protein.**

- Set up and label the required number of 1.5 mL reaction tubes and add 200 µL of “diluted DNP solution” to each (one for each standard, carbonyl control and sample) to tube #1
- Prepare a duplicate set of labeled tubes and add 1 mL ELISA buffer to each tube #2.

- Select **one option** from the following:
 - (i) For **plasma, serum or other samples containing 35-80 mg/mL protein**: Add 5 μ L of each standard, control or sample to the appropriate tube #1 containing “diluted DNP solution.” (The standards and control are at 40 mg/mL).

Mix and incubate for 45 min at room temperature. Add 5 μ L of each derivatized sample to the appropriate ELISA buffer tube #2.
 - (ii) For **samples containing 4-35 mg protein**: Add a volume of sample containing 200-300 μ g protein to the appropriate tube (to a maximum of 50 μ L for a 4 mg protein/mL sample). Dilute the standards and control 1/10 in ELISA buffer, for a protein concentration of 4 mg/mL. Add 50 μ L to the appropriate tube #1. *Mix and incubate for 45 min at room temperature.*

Add 5 μ L of each derivatized sample to the appropriate ELISA buffer tube #2.
 - (iii) For **samples containing 0.4-4mg protein**: Add a volume of sample containing 20-30 μ g protein to the appropriate tube (to a maximum of 50 μ L for a 4 mg protein/mL sample). Dilute the standards and control 1/100 in ELISA buffer, for a protein concentration of 0.4 mg/mL. Add 50 μ L to the appropriate tube #1.

Mix and incubate for 45 min at room temperature.

Add 50 μ L of each derivatised sample to the appropriate ELISA buffer tube #2.

B: The samples are now ready for ELISA Procedure (3).

C: Low Protein (<0.4 mg/mL) Procedure

Appropriate for cell extracts of biological fluids such as cerebrospinal fluid and bronchoalveolar lavage fluid.

- Determine total protein concentration of samples. If these are within 0.4-80 g/L, use procedure A. For concentrations below this range, proceed as follows.
- Initially, make a 10-fold dilution of the standards and quality controls by diluting 5 μ L with 45 μ L ELISA Buffer.
- Transfer a volume containing 20 μ g protein from each

sample to a labeled 0.5 mL tube and make all samples up to an equal volume with deionized water.

- Add 0.8 volumes of ice cold 28% (w/v) TCA (not provided), mix and leave on ice for 10 minutes.
- Centrifuge at 10000 rpm for 3 min and carefully aspirate the supernatant from the inner side of each tube **without disturbing the pellet**.
- Add 5 μ L of ELISA Buffer plus 15 μ L “diluted DNP solution” to each sample tube. Vortex thoroughly.
- Take 5 μ L of each diluted standard and control and add 15 μ L “diluted DNP solution.”
- Leave at room temperature for 45 min. To ensure that the incubation time is similar for all samples, start the timer after the first sample has been added and perform the later dilution into ELISA buffer at approximately the same speed as when adding the sample to the “diluted DNP solution.”
- Prepare complimentary set of 1.5 mL tubes for samples, standards and controls.
- Add 1 mL ELISA Buffer into each tube.
- Add 5 μ L of each DNP-treated sample to the appropriate tube and mix well.

The samples are now ready for ELISA Procedure (3).

2. **Plate Set up**

- Create a plate map indicating location of samples and standards. We recommend the samples and standards should be analyzed in triplicate (preferred) or duplicate.

3. **ELISA Procedure**

- Add 200 μ L of each sample (in ELISA Buffer) into each of the assigned ELISA plate wells. Cover the plate with sealing tape (can be cut to size if required).
- Leave plate at 4°C overnight (preferred procedure) or for 2 hours at 37°C.
- Wash plate with ELISA Buffer (5 x approx, 300 μ L per well).
- Add 250 μ L of “diluted blocking solution” per well and incubate for 30 min at room temperature.
- Wash plate with ELISA Buffer (5 x approx. 300 μ L per well).

- Add 200 μ L of “diluted anti-DNP biotin antibody” per well and incubate for 1 hour at 37°C.
- Wash plate with ELISA Buffer as above.
- Add 200 μ L of “diluted streptavidin-HRP” per well and incubate 1 hour at room temperature.
- Wash plate with ELISA Buffer as above.

4. **Color Development and Measurement**

Add 200 μ L of Chromatin reagent (I) per well and let color develop for approx. 5-20 min. at room temperature. Start the timer when the Chromatin reagent is being added with the multi-channel pipette to the first row or column and keep the time intervals between pipetting rows or columns constant. Positive wells will turn blue and the reaction can be followed at 650 nm. The reaction should continue until the highest standard K6 reaches an OD of 0.4-0.5 at 650 nm. Then stop the reaction with 100 μ L of Stopping Reagent (J) per well. This will result in a color change to yellow, increasing absorbances about threefold. Shake plate gently to mix reagents. Read absorbances at **450 nm** directly after stopping reaction.

5. **Analysis of Results**

Construct a linear **or best fit** regression standard curve by plotting the lot specific nmol/mg protein carbonyl concentration of the standards, against their adsorbances. Lot-specific standard concentrations can be found on the technical bulletin provided with your kit. An r^2 value of close to 1 should be obtained. (See example below.)

Calculate the carbonyl content of your samples (nmol/mg protein) from your standard curve by using the regression factors obtained from your standard curve (A = intercept with the y-axis, B = slope). Many plate readers can be programmed to construct a best fit standard curve and calculate sample concentrations from it. Alternatively, this can be performed manually using graph paper.

The control provided with the kit should have protein carbonyl levels similar to K4. Including this control in the assay is optional; it is intended to provide reassurance that the assay detects protein carbonyls in a sample when analyzed by the customer.

The intra- and inter-assay variation (for assays performed on the same or on different days) of samples with high carbonyls is ex-



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pected to be around 5%. Samples with low carbonyls (<0.100 nmol/mg) can have a higher inter-assay variation of about 15% because they are closer to the low end of the standard curve.

The protein carbonyl content of plasma from most healthy adults, as determined by ELISA, is below 0.1 nmol/mg. It is elevated in some disease states (please refer to the published literature for details).

References

Buss, H., Chan, T.P., Sluis, K.B., Domigan, N.M. and Winterbourn, C.C. *Protein Carbonyl Measurement By A Sensitive ELISA Method*. Free Rad. Biol. Med. (1997) Vol 23, pp 361-366.

Winterbourn, Christine C. and Buss, I. Hendrikje. *Protein Carbonyl Measurement by Enzyme-linked Immunosorbent Assay*. Methods in Enzymology. (1999) Vol 300, pp 106-111.

Buss, I.H., Darlow, B.A. and Winterbourn, C.C. *Elevated protein carbonyls and lipid peroxidation products correlating with myeloperoxidase in tracheal aspirates from premature infants*. Pediatric Research. (2000) Vol 47, pp 640-645.

Inder T., Mocatta T., Darlow, B., Spencer, C., Volpe, J.J. and Winterbourn, C. *Elevated free radical products in the cerebrospinal fluid of VLBW infants with cerebral white matter injury*. Pediatric Research. (2002) Vol 52, pp 213-218.



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NOTES



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