Protein Carbonyl Assay Kit

Catalog No.: abx257881

Size: 96 tests

Storage: Store all components in the dark at 4°C.

Application: For detection and quantification of Protein Carbonyl content in serum, plasma, tissue homogenates, hydrothorax and cell culture supernatants.

Introduction

Abbexa's Protein Carbonyl Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Protein Carbonyl content. Protein Carbonyl groups react with DNPH to form a red-brown precipitate. Once dissolved, this precipitate has an absorbance at 370 nm, which can be measured using colorimetric detection. The intensity of the color is indirectly proportional to Protein Carbonyl content, which can then be calculated.

Kit components

1. 96-well microplate
2. Homogenization medium: 2 × 50 ml
3. Sulfate Reagent: 2 vials
4. DNPH: 20 ml
5. Acid Reagent: 20 ml
6. Protein Precipitation Solution: 60 ml
7. Denaturing Buffer: 3 × 50 ml
8. Plate sealer: 2

Materials required but not provided

1. Microplate reader (370 nm)
2. Double distilled water
3. Saline (0.9 % NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. Vortex mixer
7. Water bath (37°C)

Reagents required but not provided

1. Ethyl acetate
2. Anhydrous ethanol

Protocol

A. Preparation of samples and reagents

1. Reagents

- **Sulfate Reagent solution:** Dissolve a vial of Sulphate Reagent with 3 ml of double distilled water and mix fully. Prepared Sulfate Reagent solution may be stored in the dark at 4°C for up to 3 days.
- **Ethanol-ethyl acetate solution:** Mix anhydrous ethanol and ethyl acetate at a 1:1 ratio. Prepare the required volume immediately before use.

2. Samples

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 700-1000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Wash 0.02-1 g of tissue with pre-chilled PBS (0.01M pH 7.4) and dry with absorbent paper. For each 1 g of tissue, add 9 ml of pre-chilled Homogenization Medium Mince tissues to small pieces, homogenize by hand, using a mechanical homogenizer, or by ultrasonication on ice. Centrifuge the homogenate at 3100 × g at 4°C for 10 min. Collect the supernatant on ice and assay immediately. If a precipitate appears, centrifuge again**. Immediately before use**, mix 0.45 ml of the supernatant with0.05 ml of Sulfate Reagent solution, and stand at room temperature for 10 minutes. Centrifuge at 11,000-12,000 × g for 10 minutes at 4°C, and take the supernatant for detection. Non-homogenized tissue samples may be stored safely at -80ºC for up to one month.
- **Hydrothorax:** Collect a fresh hydrothorax sample into a tube containing EDTA or heparin as the anticoagulant. Centrifuge at 10,000 × g for 10 minutes at 4°C and take the supernatant on ice for detection.
- **Cell culture supernatant:** Centrifuge for 20 min at 10,000 × g to remove any precipitates. Transfer the supernatant into a clean tube and analyse immediately or store at 4°C (up to one week), -20°C (up to one month) or -80°C (up to two months). Avoid freeze-thaw cycles. Bring samples to room temperature before carrying out the assay.

Notes:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical homogenization methods.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with double distilled water, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	8-10
Mouse serum	8-10
10% Rat liver tissue homogenate	2-3
10% Mouse brain tissue homogenate	1
Human urine	1
10% Mouse heart tissue homogenate	1
10% Fish tissue homogenate	1

B. Assay Procedure

1. Set sample and sample control tubes, and label accordingly. Each sample tube requires a sample control tube.
2. Add 0.1 ml of sample, and 0.4 ml of DNPH into the sample tubes.
3. Add 0.1 ml of sample, and 0.4 ml of Acid Reagent into the sample control tubes.
4. Mix fully, and incubate all tubes at 37°C for 30 minutes in the dark.
5. Add 0.5 ml of Protein Precipitation solution to all tubes. Mix for 1 minute, then centrifuge at 13,000-14,000 × g for 10 minutes at 4°C. Discard the supernatant and retain the pellet.
6. Add 1 ml of Ethanol-ethyl acetate solution and mix for 1 minute. Centrifuge at 13,000-14,000 × g for 10 minutes at 4°C. Discard the supernatant and retain the pellet. Repeat this process three times, or until the supernatant is no longer a yellow color.
7. Add 1.25 ml of Denaturing Buffer, mix fully, and incubate at 37°C for exactly 15 minutes.
8. Mix fully to dissolve the precipitate completely, then centrifuge at 13,000-14,000 × g for 15 minutes at 4°C.
9. Set sample and sample control wells on the 96 well microplate and label accordingly.
10. Take 300 µl of the supernatant from the sample and sample control tubes to the corresponding wells on the 96 well microplate. The protein concentration in the supernatant should be determined separately **(abx097193)**.
11. Read and record the absorbance at 370 nm with a microplate reader.

Notes:

- The Bradford method is not appropriate to determine the protein concentration. Use the BCA method (**abx097193)** or equivalent.

C. Calculation of Results

$$\text{Protein carbonyl (nmol/mg protein)} = \frac{\Delta A_1 - \Delta A_2}{\varepsilon \times d} \times \frac{f \times 10^6 \times V_2}{C_{pr} \times V_1}$$

$$= \frac{\Delta A_1 - \Delta A_2}{\varepsilon \times d} \times \frac{f \times 4.55}{C_{pr}}$$

where:

ΔA_1	$OD_{Sample} - OD_{Blank}$
ΔA_2	$OD_{Standard} - OD_{Blank}$
ε	Molar extinction coefficient of carbonyl, 22000 L/mol/cm
d	Optical path of each microplate well (0.8 cm)
V_1	Total volume (1.25 ml)
V_2	Sample volume (0.1 ml)
f	Sample dilution factor
10^6	Unit conversion (1 mol/L = 10^6 nmol/ml)
C_{pr}	Sample protein concentration (mg/L)