



Protein Carbonyl Protocol

Version: 1

Replaced by version

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Summary:

Cayman Chemical's Protein Carbonyl Colorimetric Assay Kit is a convenient colorimetric assay for the measurement of oxidized proteins. Protein samples are derivatized by making use of the reaction between 2,4-dinitrophenylhydrazine (DNPH) and protein carbonyls. Formation of a Schiff base produces the corresponding hydrazone which can be analyzed spectrophotometrically at 360-385 nm. This assay can be used to measure oxidized protein in plasma, serum, cell lysates, and tissue homogenates.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Assay Kit	Cayman	10005020
HCl		
DNPH		
TCA		
EtOH		
Ethyl Acetate		

Protocol:

1. Transfer 200 μ l of sample to two 2 ml plastic tubes. One tube will be the sample tube (S#) and the other will be the control tube (C#).
2. Add 800 μ l of DNPH to the sample tube and add 800 μ l of 2.5 M HCl to the control tube.
3. Incubate both tubes (S# & C#) in the dark at room temperature for one hour. Vortex each tube briefly every 15 minutes during the incubation.
4. Add 1 ml of 20% TCA to each tube and vortex. Place tubes on ice and incubate for five minutes.
5. Centrifuge tubes at 10,000 x g for 10 minutes at 4°C in a microcentrifuge.
6. Discard the supernatant and resuspend the pellet in 1 ml of 10% TCA. Place tubes on ice and let sit for five minutes.
7. Centrifuge tubes at 10,000 x g for 10 minutes at 4°C in a microcentrifuge.
8. Discard the supernatant and resuspend the pellet in 1 ml of (1:1) Ethanol/Ethyl Acetate mixture. Manually suspend pellet with spatula, vortex thoroughly, and centrifuge tubes at 10,000 x g for 10 minutes at 4°C in a microcentrifuge.
9. Repeat Step 8 two more times.
10. After the final wash, resuspend the protein pellets in 500 μ l of guanidine hydrochloride by vortexing.
11. Centrifuge tubes at 10,000 x g for 10 minutes at 4°C in a microcentrifuge to remove any left over debris.
12. Transfer 220 μ l of supernatant from the sample (S#) tube to two wells of the 96-well plate.
13. Transfer 220 μ l of supernatant from the control (C#) tube to two wells of the 96-well plate.
14. Measure the absorbance at a wavelength between 360-385 nm using a plate reader.

1. Calculate the average absorbance of each sample and control.
2. Subtract the average absorbance of the controls from the average absorbance of the samples. This is the Corrected Absorbance (CA).
3. Determine the concentration of the carbonyls by inserting the corrected absorbance into the following equation:

$$\text{Protein Carbonyl (nmol/ml)} = [(CA)/(*0.011 \mu\text{M}^{-1})](500 \mu\text{l}/200 \mu\text{l})$$

*The actual extinction coefficient for dinitrophenylhydrazine at 370 nm is $22,000 \text{ M}^{-1}\text{cm}^{-1}$ ($0.022 \mu\text{M}^{-1}\text{cm}^{-1}$). This value has been adjusted for the pathlength of the solution in the well.