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UC Davis - Protein Carbonyl V.2

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We use this protocol and it's working

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Abstract

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.

Materials

MATERIALS

- HCl
- DNPH
- TCA
- EtOH
- Ethyl Acetate
- Assay Kit **Cayman Chemical Company** **Catalog #**10005020

Note:

Cayman Chemical RRID:SCR_008945

Troubleshooting

1 Transfer 200 µl 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 µl of DNPH to the sample tube and add 800 µl 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 xg for 10 minutes at 4ºC 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 xg for 10 minutes at 4ºC 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 xg for 10 minutes at 4ºC in a microcentrifuge.

9 Repeat Step 8 two more times.

10 After the final wash, resuspend the protein pellets in 500µl of guanidine hydrochloride by vortexing.

11 Centriguge tubes at 10,000 xg for 10 minutes at 4ºC in a microcentrifuge to remove any left over debris.

12 Transfer 220 µl of suspernatant from the sample (S#) tube to two wells of the 96-well plate.

13 Transfer 220 µl 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.

15 **Calculation**

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:

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

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