MA-0150

Protein Carbonyl Assay

(100 wells, Colorimetric, OD 375 nm, Store at 4°C)

Introduction: Oxidative stress due to an imbalance between ROS production and antioxidant defense leads to cellular damage including the oxidation of amino acid side chains, resulting in protein carbonyls. Protein carbonylation disrupts the function and structure of proteins involved in various metabolic pathways. Protein carbonylation triggers immune responses, leading to chronic inflammation affecting things like insulin sensitivity and lipid metabolism. Elevated levels of protein carbonyls are associated with age-related diseases like neurodegenerative disorders, cardiovascular disease, and diabetes. AkrivisBio's Protein Carbonyl Assay is a simple sensitive means of determining protein carbonyls in a variety of samples with a sensitivity well below 1 nmole.

Assay Principle:

Protein carbonyls (aldehydes and related groups) react with hydrazines to form hydrazones with characteristic absorbance. Dinitrophenyl hydrazine forms adducts with absorbance at 375 nm.

Assay Components:

DNPH Solution	11 ml	Amber	MA-0150A
87% TCA Solution	3 ml	NM	MA-0150B
10% Streptozocin Solution	1 ml	Blue	MA-0150C
6 M Guanidine Solution	20 ml	WM	MA-0150D
96-Well Clear Plate	1 each	N/A	MA-0150E

User Supplied Materials:

BCA Protein Assay
Acetone

Storage and Handling Considerations:

Store at 4°C. Please read the entire protocol before performing the assay. 1.5 ml microcentrifuge tubes are very convenient containers for all reactions before transferring samples to a 96-well plate to read.

DNPH, TCA, Streptozocin, Guanidine: All solutions are ready to use as supplied. Store at 4 °C. Warm DNPH, Streptozocin and Guanidine to room temperature before use. Keep TCA on ice.

Reagents: Place 10 ml acetone **(not provided)** in freezer (-20 °C) prior to starting the procedure.

Assay Protocol:

A. DNPH Assay

1. **Standard Curve:** This assay does not use a standard curve but depends upon the extinction coefficient of DNP hydrazones (22 mM^{-1} cm^{-1}). If you use the plate provided, the depth of a 100 µl (path length) is known and the determination of carbonyl content is straightforward. If you use your own plate, you need to calibrate the plate by measuring a solution in a cuvette then transferring the solution to the plate to be used. Adding 50 – 100 – 150 – 200 – 250 µl allows the determination of the pathlength in the plate accurately. Alternatively, 100 µl depth in cm = OD 100 µl/OD cuvette.
2. **Sample Preparation:** Dissolve samples in DI H_2O and centrifuge at 16,000 X g to remove any particulates. Dilute with H_2O to ~ 10 mg/ml protein. If the protein is very dilute, it can be concentrated using a 10 kDa centrifugal filter. Use 100 µl of sample containing approximately 0.5 - 2 mg protein per assay. Include a reagent background control by using 100 µl of H_2O alone.

Note: Nucleic acids interfere with the assay. Treat samples with streptozocin (1 µl for each 10 µl of sample). Leave at room temperature for 15 minutes, then centrifuge at 16,000 X g for 5 min and transfer supernatant to a fresh tube. Read OD ratio at 280/260 nm to make sure it is > 1.

3. **Reaction:** Add 100 µl DNPH to each sample, mix and incubate 10 minutes at room temperature.
4. **Precipitate:** Add 30 µl of TCA to each sample, mix, place on ice for 5 minutes, then centrifuge at 16,000 X g for 2 minutes. Carefully aspirate supernatant without disturbing protein pellet.
5. **Wash:** Add 500 µl of ice-cold acetone to each sample and wash the pellet. 30 seconds in a sonicating bath is typically sufficient to completely disperse the pellet. Freeze at -20°C for 5 minutes, centrifuge at 16,000 X g for 2 minutes and carefully aspirate the acetone.

Note: The acetone pellet is much more friable and easily disturbed than a TCA pellet. Repeat the acetone wash step once more.

6. **Solubilize:** Add 200 µl of Guanidine solution and sonicate. Most proteins are resolubilized very easily. If your protein is resistant to solubilization, sonicate briefly then incubate the solution at 60°C for 30 minutes. Centrifuge quickly to pellet any insoluble material and transfer 100 µl of supernatant to the 96-well plate.
7. **Read:** Measure OD at ~ 375 nm in endpoint mode on a microplate reader.

Note: The BCA assay is recommended. The Bradford assay is inappropriate for this purpose since guanidine interferes.

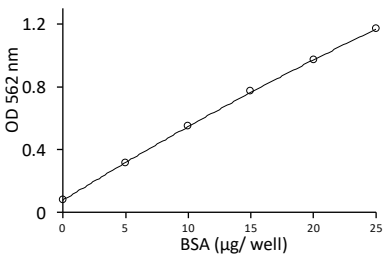
B. Transfer 5 µl of each sample to a 2nd set of wells and perform a protein assay to precisely determine the amount of protein in each sample (use BSA as standard). **Caution:** If you use more than 1 mg protein per sample, dilute it so that 25 µg protein at most, is used in the protein assay.

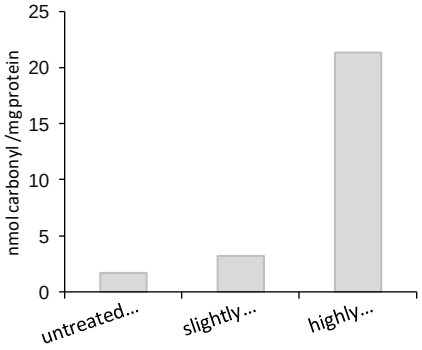
C. Calculation: Subtract the reagent background control value from all readings (The reading must be subtracted). Determine protein content of samples from protein standard curve. The BCA assay is nonlinear and is best fit by a 2nd order curve. Determine carbonyl content (C) as follows:

C = [(OD 375 nm)/6.364) x (100)] nmol/well
CP = nmol carbonyl per mg protein
= (C/P) x 1000 x D

Where:

6.364 = mM extinction coefficient using the enclosed 96 well plate
(= 22 mM^{-1} cm^{-1} * path length in well)
C = nmol Carbonyl in the sample well
P = protein from standard curve X 20 = µg/well
D = dilution or concentration step applied to sample
1000 = factor to convert µg to mg





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