Protein Carbonyls Assay Kit

Catalog Number KA3741

100 assays

Version: 03

Intended for research use only

www.abnova.com

Table of Contents

Introduction

Background

Protein carbonyl groups are an important and immediate biomarker of oxidative stress. DNPH tagging of protein carbonyls has been one of the most common measures of oxidative stress. DNP hydrazones formed from the reaction are easily quantifiable at 375 nm. Protein Carbonyls Assay Kit is designed to provide a simple and accurate method of quantifying carbonyls in protein samples. Using BSA as an example, a 1 mg (~ 15 nmol) sample has a detection limit of about 0.15 nmol carbonyl, where BSA typically contains approximately 1-3 nmol carbonyl/mg.

General Information

Materials Supplied

List of component

Component	Amount
DNPH Solution. Cap code: amber.	11 mL
100% TCA Solution. Cap code: NM.	3 mL
10% Streptozocin Solution. Cap code: blue.	1 mL
6 M Guanidine Solution. Cap code: WM.	20 mL
96-Well Clear Plate. Cap code: N/A.	1 each

Storage Instruction

- ✓ Store the kit at 4°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles. We suggest the use 1.5 mL microcentrifuge tubes for all reactions, since they are very convenient for all processing steps.
- ✓ Reagents: Place 10 mL acetone (not provided) in freezer (-20°C) prior to starting the following procedure.
- ✓ DNPH, TCA, Streptozocin, Guanidine: All solutions are ready to use as supplied. Store at 4°C in the dark. Warm the DNPH, Streptozocin and Guanidine to room temperature before use. Keep TCA on ice.

Materials Required but Not Supplied

- ✓ Protein assay reagents
- ✓ Acetone

Precautions for Use

- ✓ FOR RESEARCH USE ONLY! Not to be used on humans.

Assay Protocol

Assay Procedure

✓ DNPH Assay

1. Sample Preparation: Dissolve samples in dH_2O and centrifuge to spin down any insolubles. Dilute samples with dH_2O to approx. 10 mg/mL protein. If the protein is very dilute, it can be concentrated using a 10 kDa spin 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 dH_2O alone.
   Note: Nucleic acids interfere with the assay. Samples containing significant nucleic acid should be treated with Streptozocin (10 µL per 100 µL sample). Leave for 15 min at room temperature, spin at maximum speed for 5 min and transfer supernatant to a new tube. Check 280/260 nm ratio to make sure it is greater than 1.
2. Add 100 µL DNPH to each sample, vortex and incubate 10 min at room temperature.
3. Add 30 µL of TCA to each sample, vortex, place on ice for 5 min, spin at maximum speed for 2 min, remove and discard supernatant without disturbing pellet.
4. Add 500 µL of cold acetone to each tube and wash the pellet. 30 seconds in a sonicating bath is typically sufficient to effectively disperse the pellets. Place at -20°C for 5 min then centrifuge for 2 min and carefully remove the acetone. Caution: The acetone pellet is much more easily disturbed than the TCA pellet. Repeat the acetone wash step once more to remove free DNPH.
5. Add 200 µL of Guanidine solution and sonicate briefly. Most proteins will be resolubilized easily at this point. If your protein is resistant to resolubilization sonicate for a few seconds then let the solution sit at 60°C for 15-30 min. Spin very briefly to pellet any unsolubilized material and transfer 100 µL of each sample to the 96-well plate (included).
   Note: Must use the 96-Well plate included for accurate calculation of carbonyl content.
6. Read: Measure OD at ~ 375 nm in a microplate reader.

✓ Protein Assay: (The BCA assay (KA3718) shows minimal interference. The Bradford protein assay is inappropriate for this purpose since guanidine interferes).
Transfer 5 µL of each sample to another set of wells and perform a protein assay to precisely determine the amount of protein per sample (use BSA as the standard protein when generating your standard curve). Caution: If you are using more than 1 mg protein per sample, it must be diluted so that no more than 25 µg protein is used in the protein assay. Important to correct for any sample losses

Data Analysis

Calculation of Results

Correct background by subtracting the value derived from the reagent background control from all readings (The background reading should not be very high but must be subtracted). Determine protein content of samples from protein standard curve. The BCA assay is best fit by a 2nd order curve rather than a straight line. Determine the carbonyl content 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:

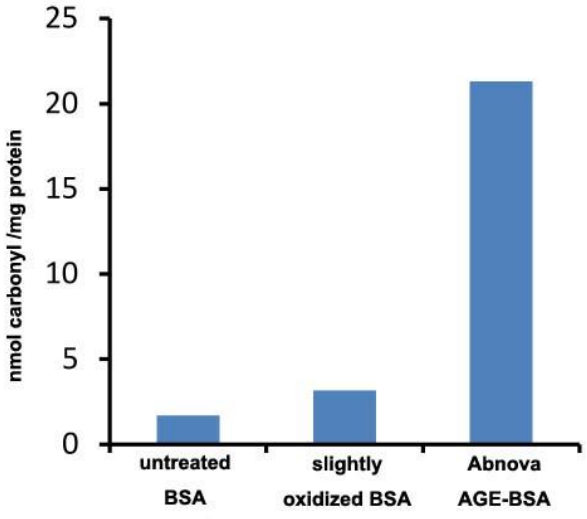
6.364 = mM extinction coefficient using the enclosed 96 well plate (= 22 mM^{-1} cm^{-1} * 0.2893 cm path length in well)

C = nmol Carbonyl in your sample well

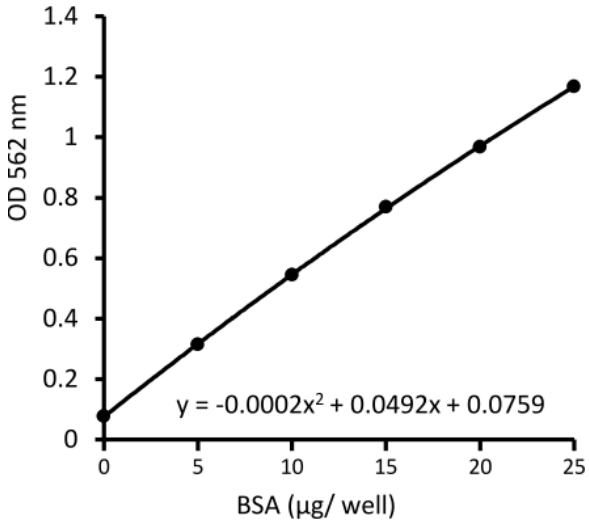
P = protein from standard curve X 20 = µg/well

D = dilution or concentration step applied to sample

1000 = factor to convert µg to mg



Representative Data Obtained Using the Protein Carbonyl Content Assay Kit



Typical Standard Curve (from BCA Assay)