Technical Bulletin

Protein Carbonyl Content Assay Kit

Catalog Number MAK094

Product Description

Oxidative stress results when the effectiveness of antioxidant defenses is insufficient to deal with the production of reactive oxygen species (ROS). ROS can induce damage to DNA, lipids, and proteins. The oxidation of proteins results in the production of stable carbonyl groups, which can be used as a measure of oxidative injury.

The Protein Carbonyl Content Assay Kit provides a simple and direct procedure for measuring carbonyl content in a variety of biological samples. Carbonyl content is determined by the derivatization of protein carbonyl groups with 2,4-dinitrophenyl-hydrazine (DNPH) leading to the formation of stable dinitrophenyl (DNP) hydrazone adducts, which can be detected spectrophotometrically at 375 nm, proportional to the carbonyls present.

The limit of detection for this kit will vary depending upon the nature of the protein being tested. With bovine serum albumin (BSA), this kit can detect carbonyl levels of ~0.15 nmole of carbonyls/mg of BSA.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- DNPH Solution — 11 mL
  Catalog Number MAK094A
- 87% TCA Solution — 3 mL
  Catalog Number MAK094B
- 10% Streptozocin Solution — 1 mL
  Catalog Number MAK094C
- 6 M Guanidine Solution — 20 mL
  Catalog Number MAK094D
- 96 Well Clear Plate — 1 ea
  Catalog Number MAK094E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Acetone
  (Catalog Number 534064 or equivalent)
- Bicinchoninic Acid Kit for Protein Determination
  (Catalog Number BCA1 or QPBCA, or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at 2–8 °C, protected from light.

Preparation Instructions

Briefly centrifuge vials prior to opening. Use purified water for the preparation of reagents and samples. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Acetone: Place 10 mL of acetone in –20 °C freezer prior to start of assay.

DNPH Solution, 10% Streptozocin Solution, and 6 M Guanidine Solution: Allow reagents to come to room temperature prior to use. Store at 2–8 °C, protected from light.

87% TCA Solution: Keep on ice while in use. Store at 2–8 °C, protected from light

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Samples should be dissolved in purified water and centrifuged to remove any insoluble material. Dilute samples to a protein concentration of ~10 mg/mL. Each assay well requires 100 µL of Sample containing 0.5–2.0 mg of protein per assay.

Add 100 µL of purified water to a well to serve as a Reagent Background Control.

Note: Nucleic acids will interfere with the assay. If Samples contain significant amounts of nucleic acids, treat Samples with 10 µL of the 10% Streptozocin solution per 100 µL of Sample. Incubate at room temperature for 15 minutes, centrifuge at 13,000 × *g* for 5 minutes, and then transfer supernatant to a new tube.

For unknown Samples, it is suggested to test several sample volumes to make sure the readings are within the Standard curve range. Bring each well to a final volume of 100 µL with purified water.

DNPH Assay Reaction

1. Add 100 µL of DNPH Solution to each Sample. Vortex and incubate for 10 minutes at room temperature.
2. Add 30 µL of the 87% TCA Solution to each Sample. Vortex and incubate on ice for 5 minutes. Centrifuge Samples at 13,000 × *g* for 2 minutes. Remove supernatant being careful not to disturb the pellet.
3. Add 500 µL of ice-cold acetone (not included) to each pellet and place in a sonication bath for 30 seconds. Incubate at –20 °C for 5 minutes and then centrifuge samples at 13,000 × *g* for 2 minutes. Carefully remove acetone from pellet. Remove acetone once more to remove free DNPH.

   Note: The acetone pellet is much more easily disturbed than the TCA pellet.

4. Add 200 µL of 6 M Guanidine Solution to pellet and sonicate briefly. Most proteins will resolubilize easily in the Guanidine Solution. Transfer 100 µL of each Sample to the 96 well plate.

   Note: If proteins are resistant to resolublization, sonicate for ~5 seconds and then incubate at 60 °C for 15-30 minutes. Spin briefly to pellet any insolubilized material. Transfer 100 µL of each sample to the 96-well plate.

5. Measure the absorbance at 375 nm (A_{375}).

Protein Assay Reaction

Transfer 5 µL of Sample to another set of wells and perform a protein assay to determine the amount of protein per sample. Generate a protein standard curve according to assay protocols. It is recommended to use bovine standard albumin for the Protein Standard curve.

Note: The Bradford Protein Assay is **not** appropriate for this assay due to interference from the guanidine in the test samples. It is recommended to use the Bicinchoninic Acid (BCA) assay to measure protein in these samples.

Results

1. Correct for the DNPH assay background by subtracting the Reagent Background Control A_{375} from all A_{375} readings.
2. Determine the protein content from the Protein assay Standard curve.
   Note: A new protein standard curve must be set up each time the assay is run.
3. Determine the carbonyl content as follows:

C (nmole/well) =

$$\frac{A_{375}}{6.364}\ x\ 100$$

where

6.364 = Millimolar extinction coefficient (ε^{mM} = 22 mM^{-1} cm^{-1}) times 0.2893 cm pathlength in a well for the enclosed 96-well plate

[6.364 = ε^{mM} (22 mM^{-1} cm^{-1}) × 0.2893 cm]

100 = Total volume (V) in well (µL)

CP (nmole carbonyl/mg protein) =

$$\frac{C}{P}\ x\ 1000\ x\ DF$$

where

C = Amount of carbonyl in sample well (nmole/well)

P = Amount of protein from standard well × 20 = µg/well. Conversion factor of 20 is from 5 µL Sample used for Protein assay to 100 µL Sample used for Carbonyl assay.

DF = Dilution factor for Sample. DF = 1 for undiluted samples.

1,000 = Conversion factor (µg to mg)

Figure 1.
Typical Standard Curve (from BCA Assay)

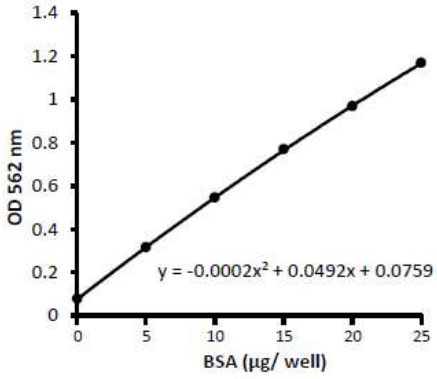
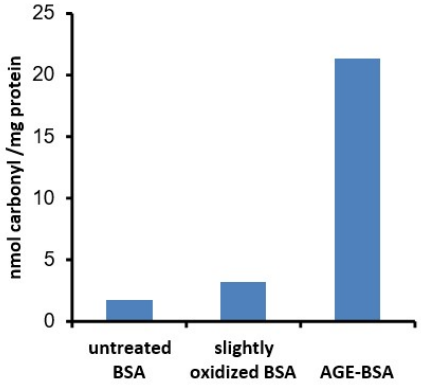


Figure 2.
Representative Data Obtained Using the Protein Carbonyl Content Assay Kit



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