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Product Information

Protein Carbonyl Content Assay Kit

Catalog Number **MAK094**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

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-dinitrophenylhydrazine (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 assays in 96 well plates.

DNPH Solution Catalog Number MAK094A	11 mL
100% TCA Solution Catalog Number MAK094B	3 mL
10% Streptozocin Solution Catalog Number MAK094C	1 mL
6 M Guanidine Solution Catalog Number MAK094D	20 mL
96 Well Clear Plate Catalog Number MAK094E	1 ea

Reagents and Equipment Required but Not Provided.

- Spectrophotometric multiwell plate reader capable of reading at 375 nm and 562 nm
- 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.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure 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 before use. Store at 2–8 °C, protected from light.

100% TCA Solution – Keep on ice while in use. Store at 2–8 °C, protected from light.

Storage/Stability

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

Procedures
All samples and standards should be run in duplicate.

Sample Preparation
Samples should be dissolved in ultrapure 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 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 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 100% 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 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 unsolubilized material. Transfer 100 µL of each sample to the 96 well plate.

5. Measure 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 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

Calculations

Correct for the DNPH assay background by subtracting the reagent background control from all readings.

Determine the protein content from the protein assay standard curve.

Note: A new standard curve must be set up each time the assay is run.

Carbonyl Content

Amount of carbonyl in sample well

$$C\ (\text{nmole/well}) = (A_{375}/6.364) \times 100$$

6.364 = Millimolar extinction coefficient (ε^{mM} = 22 mM^{-1} cm^{-1}) times 0.2893 cm pathlength in a well for 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 = (C/P) × 1,000 × D

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

P = Amount of protein from standard well × 20 = μg/well

D = dilution or concentration of sample

1,000 = conversion factor (μg to mg)

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	Use plate that came with assay kit
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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