Protein Carbonylation Assay Kit

KB-03-008

100 tests (96 well plate)

BQCkit

A brand of BioQuChem

Index

All chemicals should be handled with care

- This kit is for R&D use only

Introduction

Oxidative stress may cause reversible or irreversible changes in proteins. Such changes are meant to modulate protein function (redox regulation) or protect against irreversible damage that causes the inactive proteins to accumulate or become degraded.

Carbonylation, an irreversible oxidative damage, involves the oxidation of side chains of amino acids to aldehydes or ketones. Lysine, arginine, proline, and threonine side-chains can be oxidatively converted to reactive aldehyde or ketone groups causing inactivation, crosslinking or breakdown of proteins.

Protein carbonylation can be detected and quantified at the global level in proteins and protein mixtures using derivatization of carbonyl groups with hidrazines followed by spectrophotometric measurement.

Materials

BQCkit Protein Carbonylation Assay Kit *KB-03-008 100 tests* contains:

Product	Quantity	Storage
Reagent A	2 vials	4ºC
Reagent B	2 vials	4ºC
Reagent C	2 vials	4ºC
Reagent D	1 vial	4ºC
Reagent E	2 vials	4ºC
Reagent F	1 vial (powder)	4ºC

Assay Principle

Protein carbonylation occurs providing an integrated assessment of oxidative damage.

The most employed method for evaluation of the content of carbonylated proteins is based on 2,4-dinitrophenylhydrazine (DNPH) and was originally developed by Levine et al.. This molecule reacts with carbonyl groups leading to the formation of the stable 2,4-dinitrophenylhydrazone (Figure 1). The dinitrophenyl group (DNP) can be detected and quantified spectrophotometrically because it is characterized by a typical absorption spectrum with a maximum at 365–375 nm.

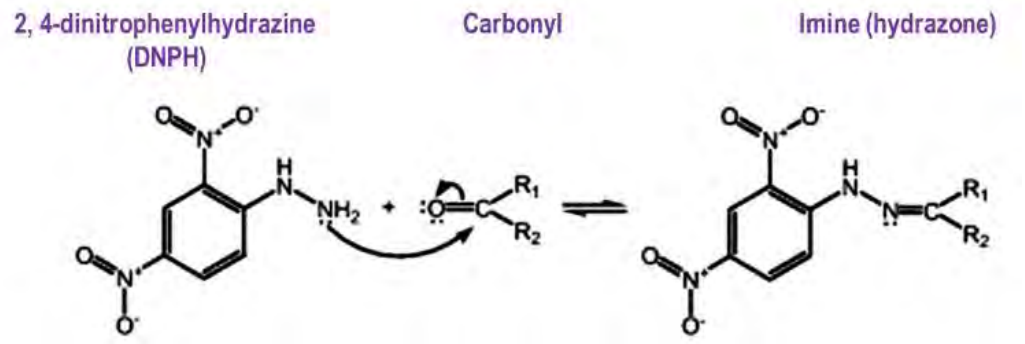

Figure 1. *DNPH reaction with carbonyl species. Modified from Uchiyama et al.*

Reagent Preparation

- Add exactly 1.57 mL of purified water to Reagent F and mix thoroughly. This reagent must be freshly prepared.

- Put Reagent D at -20ºC before starting the assay.

Sample Preparation

- It is recommended to assay the samples in duplicate.
- Calculate the protein concentration of the samples using Bradford Reagent and Protein Standard. For this purpose, Bioquochem has available the Fast Protein Quantification Kit (Bradford method) with the reference KB-03-003.
- Samples should be diluted with water to a protein concentration of 1 mg/mL. If the protein is very dilute, it can be concentrated using a 10 kDa spin filter.
- Removal of nucleic acids. Nucleic acids are carbonyl positive and may erroneously contribute to a higher estimation of carbonyls. To know if nucleic acids are interfering with your samples, check the relation A280 nm/A260 nm of your samples.

  If this ratio is <1:

  - Add 15 µL of Reagent F to 200 µL of your sample.
  - Incubate the samples at room temperature for 15 minutes and then, centrifuge them at 10,000 x *g* for 5 minutes at 4°C.
  - Transfer supernatant to a new tube.
  - Check 280/260 nm ratio to make sure it is greater than 1.

Assay Protocol

1. Transfer 100 µL of sample containing 1 mg of protein to a vial. This will be the SAMPLE TUBE.
2. Transfer 100 µL of sample containing 1 mg of protein to a vial. This will be the CONTROL TUBE.
3. Add 100 µL of Reagent A to the sample tube and 100 µL of Reagent B to the control tube.
4. Incubate both tubes in the dark at room temperature for 1h, with vortex-mixing every 15 minutes.
5. Add a volume of 50 µL of Reagent C to each tube. Vortex thoroughly and place them on ice for 5 min.
6. Centrifuge samples at 10,000 x g for 5 min at 4°C.Then, remove and discard supernatant without disturbing pellet.
7. Add 500 µL of Reagent D to each tube in order to wash the pellet. Place them at -20°C for 5 min and then centrifuge for 5 min.
8. Once centrifugated, remove carefully undiscard supernatant. Caution: This pellet is much more easily disturbed than the previous pellet.
9. Repeat the wash step at least twice until supernatants are completely transparent.

10. After the final wash, resuspend the protein pellets in 200 µL of Reagent E incubating at room temperature for 5 min with vortex mixing.

11. Once protein pellets are completely dissolved, transfer 100 µL of the supernatant of each sample to the 96-well plate.

12. Carbonyl content can be determined from the peak absorbance at ~375 nm using a molar absorption coefficient of 22,000 $M^{-1}cm^{-1}$.

13. The CONTROL TUBE should be used as a blank in order to subtract intrinsic protein absorbance to the absorbance of specific adducts.

14. Bradford Assay: Proteins are lost during the washing steps so protein levels are determined again on the final pellet after the washes.

Data Analysis

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.
3. Determine the concentration of the carbonyl content by inserting the Corrected Absorbance into the following equation:

$$CC\ (nmol/well) = [(A\ 375\ nm)/22\ mM^{-1}cm^{-1} \times 0.2893\ cm] \times (100\ \mu L)$$

$$CC\ per\ mg\ protein\ (nmol) = (CC\ /P) \times 1000 \times D$$

Where:

A375 nm = Corrected Absorbance
ε= 22 mM^{-1} cm
b= 0.2893 cm path length in 96 well plate
CC= Carbonyl Content in your sample
P= Protein from standard curve, µg (Bradford assay)
1000= the factor to convert µg to mg
D= Dilution or concentration step applied to sample.

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Expiration date: 1 year from the date of delivery

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