BQC

REDOX TECHNOLOGIES

KB03044
Protein Carbonyl Assay Kit

96 well plate
100/200/400 tests

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1. General information

PRECAUTIONS

Please read this manual carefully before beginning the assay.

This product is designed for **research use only**. It is not approved for human or animal use or clinical diagnosis. All chemicals should be handled with care and in accordance with laboratory safety practices. It is recommended to use basic Personal Protective Equipment.

Do not use after the expiration date stated on the packaging.

Do not mix or substitute reagents or materials from other kit batches or vendors.

For the **material safety data sheet** (MSDS) please contact us at info@bioquochem.com

TECHNICAL RECOMMENDATIONS

Store reagents as indicated in **Materials and storage** section.

Be sure to keep the bottle capped when not in use.

Let the components reach room temperature (RT) before use.

Immediately before use, gently invert and rotate reagent bottles several times to mix the contents thoroughly.

Avoid foaming or bubbles when mixing or reconstituting components.

Avoid cross contamination of samples or reagents by changing pipette tips between sample, standard and reagent additions.

Be sure to use the optimal microplate for the assay. Flat bottom transparent microplates for UV/VIS applications, and black microplates for fluorescence measurements.

2. Technical specifications

Available sizes

100/200/400 tests

Required sample volume

60 µL/test

Compatible samples

Biological fluids, tissue homogenates, cells, bacteria and other biological samples

Type of detection

Colorimetric (370 nm)

3. Materials and storage

MATERIALS SUPPLIED

Item	No. Tests	Units	Storage
Reagent A	100	1	4 °C
	200	2	
	400	4	
Reagent B	100	1	4 °C
	200	2	
	400	4	
Reagent C	100	1	4 °C
	200	2	
	400	4	
Reagent D	100	1	-20 °C
	200	2	
	400	4	
Reagent E	100	1	4 °C
	200	2	
	400	4	
Reagent F	100	1	4 °C
	200	2	
	400	4	
Reagent G	100	1	4 °C
	200	2	
	400	4	
Reagent H	100	1	-20 °C
	200	2	
	400	4	
Transparent 96-Well Microplate	100	1	RT
	200	2	
	400	4	

MATERIALS NEEDED BUT NOT SUPPLIED

- Double distilled water (ddH_2O) as Milli-Q Ultrapure Water
- Labware materials (micropipettes, tubes, stirring/mixing equipment)
- Incubator, refrigerated microcentrifuge
- Colorimetric microplate reader – equipped with filter for OD 370 nm

STORAGE CONDITIONS

On receipt, store kit components as indicated above. Under these conditions, the reagents are stable in the original packaging until the expiration date stated on the outside of the box. **Reagent A, G and H** are light sensitive and should be stored in the dark.

4. Introduction

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

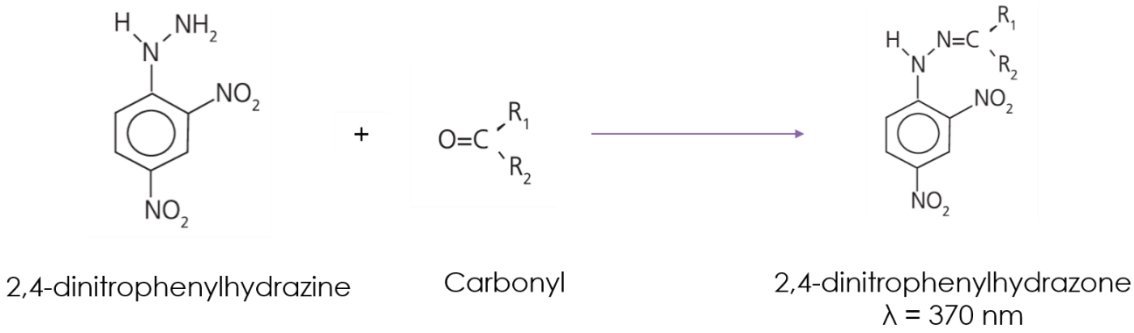
Carbonylation, an irreversible oxidative damage, involves the oxidation of side chains of amino acids to aldehydes or ketones. Lysine, arginine, proline, and threonine sidechains can be oxidatively converted to reactive aldehyde or ketone groups causing inactivation, crosslinking or breakdown of proteins.

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

BQC Protein Carbonyl Assay Kit is designed to detect carbonylated proteins in a simple and accurate test with high reproducibility.

5. Assay principle

This Assay Kit is based on the 2,4-dinitrophenylhydrazine (DNPH) method, a widely used method for the determination of protein carbonylation. This method utilizes the reaction of carbonyl groups with DNPH molecule leading to the formation of protein-bound 2,4-dinitrophenylhydrazone with absorbance at 370 nm. Dinitrophenylhydrazone can be detected and quantified spectrophotometrically because it is characterized by a typical absorption spectrum with a maximum at 365–375 nm.



Principle of Protein Carbonyl Assay Kit

6. Assay preparation

REAGENT PREPARATION

All assay reagents not listed below are ready to use as supplied. Allow the reagents to reach room temperature before use.

CAUTION: Working Solutions must be prepared immediately before use.

For animal and plant tissues prepare R.G. Working Solution: for ten samples add 0.1 g of Reagent G to 1 mL of deionized water and mix thoroughly. Store at 4 °C in the dark until used for **Sample preparation.**

PLATE SET UP

BQC recommends running the samples and controls at least in duplicate (triplicate recommended). There is no specific pattern for using the wells on the plate. A proposed layout of samples (S) and sample blanks (SB) to be measured in duplicate is shown below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S5	S5	S9	S9	S13	S13	S17	S17	S21	S21
B	SB1	SB1	SB5	SB5	SB9	SB9	SB13	SB13	SB17	SB17	SB21	SB21
C	S2	S2	S6	S6	S10	S10	S14	S14	S18	S18	S22	S22
D	SB2	SB2	SB6	SB6	SB10	SB10	SB14	SB14	SB18	SB18	SB22	SB22
E	S3	S3	S7	S7	S11	S11	S15	S15	S19	S19	S23	S23
F	SB3	SB3	SB7	SB7	SB11	SB11	SB15	SB15	SB19	SB19	SB23	SB23
G	S4	S4	S8	S8	S12	S12	S16	S16	S20	S20	S24	S24
H	SB4	SB	SB8	SB8	SB12	SB12	SB16	SB16	SB20	SB20	SB24	SB24

Example of plate layout for the Protein Carbonyl Assay Kit

7. Sample preparation

The following sample preparation protocols are intended as a guide only. The optimal conditions for sample preparation must be determined by the end user. It is recommended to use fresh samples. If it is not possible, aliquot and store samples appropriately with minimal freeze/thawing.

Protein Carbonyl Assay Kit can be used to determine carbonylation in serum, plasma, tissue homogenates and cell lysates.

NOTE: If results are needed in sample protein concentration:

- **Calculate the total protein concentration** of samples using **BQC Bradford (KB03003), or BCA (KB03005) Assay Kits.** If sample contains guanidine, use BCA Assay Kit because guanidine interferes with Bradford Assay Kit.
- Samples should be diluted with ddH_2O to obtain **a protein concentration between 10-40 mg/mL.** If the sample is highly diluted, it can be concentrated using a 10 kDa Amicon. For each replicate, a total volume of 120 µL will be needed.

CAUTION: 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 A_{280nm}/A_{260nm} of your samples. This ratio should be higher than 1.

If this ratio is <1:

- Add **15 µL of Reagent H** to 200 µL of your sample.
- Incubate the samples at **room temperature for 15 minutes** and then, centrifuge them at 10000 g for 5 minutes at 4 °C. Transfer supernatant to a new tube.
- Check A_{280nm}/A_{260nm} ratio to make sure it is greater than 1.

Serum and Plasma. Can be tested directly.

Animal and Plant Tissues. Weigh 0.1 g of tissue, mix with 1 mL of buffer and homogenize on ice. Centrifuge at 4000 g for 10 minutes at 4 °C. Take the supernatant, add 0.1 mL of **R.G. Working Solution**, keep at RT for 10 minutes, centrifuge at 10000 g for 10 minutes at 4 °C. Use supernatant and place on ice to be tested.

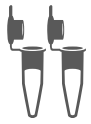
Cells and bacteria. Collect $5 \cdot 10^6$ cells, wash with cold PBS and discard supernatant after centrifugation. Add 1 mL of buffer to ultrasonically disrupt cells for 5 minutes. Centrifuge at 10000 g for 10 minutes at 4 °C. Use supernatant for the assay and place it on ice to be tested.

Reagents and materials required for sample preparation are not supplied with the kit. Before doing sample preparation, consider the volume of sample required per test; see **Technical specifications** section.

Make sure that interfering substances present in the sample do not give a significant background. Run proper blanks as necessary. It is recommended to assay different sample dilutions to ensure the values fall within the standard curve.

8. Assay protocol

Prepare and mix all reagents thoroughly before use. Each sample or blank sample should be assayed at least in duplicate.

1 

- Transfer **60 µL** of **sample** to a vial (**sample** tubes)
- Transfer **60 µL** of **sample** to another vial (**blank sample** tubes)

2 

- **Sample** tubes: Add **120 µL** of **Reagent A**
- **Blank sample** tubes: Add **120 µL** of **Reagent B**

3 

Incubate sample and **blank sample** tubes **in the dark at 37 °C for 1 h**

4 

Add **150 µL** of **Reagent C** to each tube and keep it still for **5 minutes**

5 

Centrifuge at **12,000 g for 15 minutes at 4°C**. Then, remove and discard supernatant

6 

Add **150 µL** of **Reagent D** to each tube. Mix with vortex

7 

Add **150 µL** of **Reagent F** to each tube. Mix with vortex

8 

Centrifuge at **12,000 g for 10 minutes at 4°C**. Once centrifuged, remove carefully supernatant

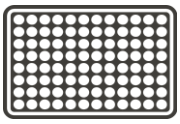
> **NOTE:** This pellet is much more easily disturbed

9 

Repeat the washing step (steps 6, 7 and 8) three times

10  **Resuspend** the pellets in **300 µL of Reagent E**, mix well and incubate **at 37 °C for 15 minutes** with vortex mixing until the pellet is completely dissolved

11  **Centrifuge** at **12,000 g for 15 minutes at 4 °C**. Keep the supernatant

12  Set up the plate design

13  Transfer **200 µL** of the supernatant from each tube into a 96-well plate

14  Read the **absorbance** of all wells at **370 nm**

CAUTION: Proteins are lost during the washing steps, so protein levels should be determined again on the final pellet after the washing steps. Use the remaining liquid in the tubes to measure the protein concentration with BQC BCA Assay or another Protein Quantification Assay Kit.

If you need to **adapt this kit** for another form of the assay (for example cuvette), **contact us at** info@bioquochem.com

9. Data analysis

ANALYSIS OF THE SAMPLES

- Calculate the average absorbance of the samples and blank samples.
- Subtract the average absorbance of the blank samples from the average absorbance of the samples to obtain the blank-corrected absorbance of the samples (A_s).
- Determine the concentration of the carbonyl content by inserting the blank-corrected absorbance (A_s) into one of the following formulas:

Calculated by fresh weight of samples

$$\text{Carbonyl content (µmol/g fresh weight)} = = \frac{0.454 \times A_s}{W}$$

Calculated by protein concentration

$$\text{Carbonyl content (µmol/mg protein)} = \frac{0.454 \times A_s}{C_p}$$

Calculated by cells or bacteria number

$$\text{Carbonyl content (µmol/}10^4\text{ cells)} = \frac{0.454 \times A_s}{500}$$

Calculated by volume of liquid samples

$$\text{Carbonyl content (µmol/mL)} = 0.454 \times A_s$$

Where W is the sample weight (g) and Cp is the sample protein concentration (mg/mL).

When working with diluted samples the concentration values obtained must be multiplied by the dilution factor to obtain the content of the undiluted sample.

10. Troubleshooting

This troubleshooting table provides potential sources and solutions for common problems observed with BQC Assay Kits. **The problems listed below could occur when using any BQC Assay Kit**. They are not specific for this assay kit.

Problem	Possible Cause	Recommended Solution
Wells have color but there is no reading	Plate read at incorrect wavelength	Check the wavelength used in the assay
	Incorrect microplate	Use the correct microplate for your application UV/Vis: transparent Fluorescence: black wells/transparent bottom
Standard readings do not follow a linear pattern	Pipetting errors in preparation of standards	Avoid pipetting small volumes (<5 µL) Be careful not to splash from well to well
	Air bubbles formed in well(s)	Use reverse pipetting technique
	Standard stock is at incorrect concentration	Always refer to dilutions described in **Assay preparation**
	Improperly thawed reagents	Thaw all components completely and mix well before use
	Use of improperly stored reagents	Store the components appropriately Use fresh components from the standard curve
	Incorrect incubation times or temperatures	Refer to **Assay protocol**
Dispersion of standard and sample readings	Pipetting errors	Avoid pipetting small volumes (<5 µL) Be careful not to splash from well to well
	Air bubbles formed in well(s)	Use reverse pipetting technique

Problem	Possible Cause	Recommended Solution
Sample erratic values	Samples contain interfering substances	Dilute sample further (if possible)
	Inappropriately stored samples or samples used after multiple freeze-thaw cycles	Use fresh samples or store appropriately until use
	Samples not deproteinized	Use an appropriate deproteinization protocol
	Cells/Tissue samples not homogenized completely	Repeat the sample homogenization
	Inappropriate sample dilution buffer	Refer to **Assay preparation**
Sample reading fall outside the detection range	Samples are too diluted/concentrated No analyte/activity is observed in the sample	Re-assay using different sample dilutions

STILL HAVING PROBLEMS?

Contact BQC if you have any further questions, our team will be pleased to help you:

	Phone	+ 34 985 26 92 92
	E-mail	info@bioquochem.com
	Business hours	Monday-Thursday: 8.30 to 17.00 (CEST) Friday: 8.00 to 15.00 (CEST)

11. Additional information

Protein Carbonyl Determination Assay Kit is a simple assay for determining protein carbonyls in a wide variety of samples.

Nucleic acids have been reported to interfere with this assay. If present, follow the protocol described in **Sample preparation** section or if it is not possible, dilute sample further. If the sample contains guanidine, avoid using Bradford Protein Assay Kit to determine protein concentration since guanidine interferes in the assay.

If unexpected results are obtained running your samples, please contact us at info@bioquochem.com

12. Related products

More products available on bioquochem.com

Reference	Product
KB03031	Protein Concentration Assay
KB03003	Bradford Protein Quantification Assay
KB03005	BCA Protein Quantification Assay

13. Warranties and limitation of liability

BQC shall not in any event be liable for incidental, consequential or special damages of any kind resulting from any use or failure of the products, even if BQC has been advised of the possibility of such damage including, without limitation, liability for loss of use, loss of work in progress, downtime, loss of revenue or profits, failure to realize savings, loss of products of buyer or other use or any liability of buyer to a third party on account of such loss, or for any labor or any other expense, damage or loss occasioned by such product including personal injury or property damage is caused by BQC´s gross negligence. Any and all liability of BQC hereunder shall be limited to the amounts paid by the buyer for the product.

Buyer´s exclusive remedy and BQC´s sole liability hereunder shall be limited to a refund of the purchase price, or the replacement of all material that does not meet our specifications.

Said refund or replacement is conditioned on buyer giving written notice to BQC within 30 days of shipment.

Expiration date: 1 year from the date of fabrication. Expiration date is indicated on the outside of the box.

For further details, please refer to our website **bioquochem.com**

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