Version 2 Last updated 5 February 2024

ab235631
Protein Carbonyl Content Assay Kit (Fluorometric)

For the measurement of carbonyl content in biological fluids such as serum, plasma, and cell lysate.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

Protein Carbonyl Content Assay Kit (Fluorometric) (ab235631) uses Fluoroscein-5-Thiosemicarbazide (FTC), a fluorescent probe which covalently reacts with oxidized residues (i.e. Cysteine, Lysine, Arginine, Histidine and Aspartic Acid) on proteins. The protein carbonyl content is determined by the generation of a stable fluorometric signal (Ex/Em 485/535 nm) and compared to the protein concentration determined in the BCA Assay to quantitate nmoles of carbonyl/mg protein. The kit is simple, requires no harsh chemicals, can quantitate carbonyls in serum or plasma and produces more reliable and reproducible results than the comparable colorimetric assays. It can detect carbonyl groups in samples with protein concentrations as low as 1 mg/mL.

Prepare samples.

Prepare Reaction Mix. Add to sample and allow fluorophore to react with sample overnight at room temperature.

Precipitate proteins by adding 20% ice-cold TCA. Wash pellet with Isopropanol. Resuspend pellet with 50 µL Guanidine Solution/6 M Guanidine and incubate samples on a heat block at 50°C for 1-2 hours to improve solubility of pellet.

When pellet is completely dissolved, add 450 µL of Carbonyl Sample Dilution Buffer/Sample Dilution Buffer.

Determine protein concentration in solubilized pellet. Add 100 µL of each solubilized pellet sample/well in a 96-well clear microplate.

Prepare FTC Standard Curve.

Measure fluorescence of all standards, samples and background controls (Ex/Em 485/535 nm).

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Protein Carbonyl Assay Buffer	20 mL	-20°C	-20°C
100% TCA Solution	20 mL	-20°C	-20°C
Streptozocin Solution/10% Streptozotocin Solution	1 mL	-20°C	-80°C
Guanidine Solution/6 M Guanidine Solution	20 mL	-20°C	-20°C
Carbonyl Sample Dilution Buffer/Sample Dilution Buffer	50 mL	-20°C	-20°C
Fluoroscein-5-Thiosemicarbazide (FTC)/FTC (10 mM in DMSO)	200 µL	-20°C	-20°C
AGE-BSA Positive Control Protein/Positive Control Protein (10 mg/mL)	250 µL	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multi-well fluorescence microplate reader.
- 96-well clear microtiter plates with flat bottom.
- Isopropanol.
- BCA Protein Assay Kit, or BCA Protein Assay Kit-Reducing Agent Compatible (ab207003).

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Protein Carbonyl Assay Buffer

1. Ready to use as supplied.
2. Warm to room temperature before use.
3. Store at -20°C in dark.

5.2 100% TCA Solution

1. Upon receipt of kit, inspect bottle.
2. If precipitation is observed, vortex the bottle or warm the content at 37°C until it completely dissolves.
3. Before use dilute in dH_2O to 20% TCA and keep on ice.

5.3 Streptozocin Solution/10% Streptozotocin Solution

1. Ready to use as supplied.
2. Warm to room temperature before use.
3. Store at -20°C in dark.

5.4 Guanidine Solution/6 M Guanidine Solution

1. Ready to use as supplied.
2. Upon receipt of kit, inspect bottle.
3. If precipitation is observed, vortex the bottle or warm the content at 37°C until it completely dissolves.
4. Warm to room temperature before use.

5.5 Carbonyl Sample Dilution Buffer/Sample Dilution Buffer

1. Ready to use as supplied.
2. Warm to room temperature before use.
3. Store at -20°C in dark.

5.6 Fluoroscein-5-Thiosemicarbazide (FTC)/FTC (10 mM in DMSO)

1. Ready to use as supplied.
2. Warm to room temperature before use.
3. Store at -20°C in dark.

5.7 AGE-BSA Positive Control Protein/Positive Control Protein (10 mg/mL)

1. Ready to use as supplied.
2. Warm to room temperature to liquefy completely before use.
3. Store at -20°C in dark.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Prepare a 25 µM solution of Fluoroscein-5-Thiosemicarbazide (FTC)/FTC by adding 2 µL of stock Fluoroscein-5-Thiosemicarbazide (FTC)/FTC (10 mM) to 798 µL of Protein Carbonyl Assay Buffer to create a working standard.
2. Using 25 µM solution of Fluoroscein-5-Thiosemicarbazide (FTC)/FTC, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard#	Fluoroscein-5-Thiosemicarbazide (FTC)/FTC Standard (µL)	Protein Carbonyl Assay Buffer (µL)	Final volume standard in well (µL)	End amount of Fluoroscein-5-Thiosemicarbazide (FTC)/FTC standard in well (pmoles/well)
1	0	300	300	0
2	6	294	300	50
3	12	288	300	100
4	18	282	300	150
5	24	276	300	200
6	30	270	300	250

Each dilution has enough standard to set up duplicate readings (2 x 100 µL).

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

7.1 Serum:

1. The assay can be used on serum, plasma as well as other biological fluids when applicable.
2. Use Protein Carbonyl Assay Buffer to adjust sample concentrations to 1-10 mg/mL prior to performing the assay.
3. Dilute samples in Protein Carbonyl Assay Buffer and centrifuge to remove any insolubles.
4. Use 50 µL of sample in a centrifuge tube.

Δ Note: Protein samples lower than 1 mg/ml should be concentrated with a 10 kD spin filter (ab93349).

Δ Note: Nucleic acids can erroneously contribute to higher estimation of carbonyls. Samples containing significant nucleic acid content should be treated with Streptozocin Solution/Streptozotocin (10 µL per 100 µL sample). Incubate for 15 minutes at room temperature, centrifuge for 5 minutes x 10000 x *g* and transfer supernatant to a new tube. Measure 280/260 nm ratio to make sure it is greater than 1.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 Reagent Background Control and Positive Control:

1. Prepare a reagent background control using 50 µL of Protein Carbonyl Assay Buffer.
2. For Positive Control, dilute AGE-BSA Positive Control Protein/Positive Control Protein to 5 mg/ml (50 µL of Positive Control Protein with 50 µL of Protein Carbonyl Assay Buffer). Add 50 µL of 5 mg/mL AGE-BSA Positive Control Protein/Positive Control Protein to an unused 1.5 mL centrifuge tube.

8.2 Reaction Mix:

1. Dilute Fluoroscein-5-Thiosemicarbazide (FTC)/Fluorophore (FTC) 50 times with Protein Carbonyl Assay Buffer (i.e. for 5 samples add 5 µL of Fluoroscein-5-Thiosemicarbazide (FTC)/FTC to 245 µL of Protein Carbonyl Assay Buffer).
2. Prepare sufficient reagent for the number of samples to be assayed (50 µL/assay).
3. Add 50 µL of 0.2 mM Fluoroscein-5-Thiosemicarbazide (FTC)/FTC to each 50 µL sample in a 1.5 mL centrifuge tube.
4. Allow fluorophore to react with sample overnight at RT, protect from light.

8.3 Quantify Carbonyl Content:

1. On the next day, precipitate proteins by adding 200 µL of 20% ice-cold TCA/centrifuge tube.
2. Incubate mixture 10 minutes on ice.
3. Centrifuge for 10 minutes at 10000 x *g*, 4°C. Remove supernatant.
4. Wash pellet with 200 µL of 100% ice-cold isopropanol (not provided).

5. Manually break-up pellet with pipette tip. (Small particles at this step improve solubility when Guanidine Solution/6 M Guanidine added).
6. Centrifuge samples (as above), remove supernatant.
7. Repeat wash step for a total of three times and, allow pellet to air dry at room temperature (this may take 1 hour).
8. Resuspend pellet with 50 µL of Guanidine Solution/6 M Guanidine and incubate samples on a heat block at 50°C for 1-2 hours to improve solubility of pellet.
9. Cool down sample to room temperature.
10. When pellet is completely dissolved, add 450 µL of Carbonyl Sample Dilution Buffer/Sample Dilution Buffer.
11. Determine protein concentration in solubilized pellet (i.e. BCA Assay).
12. Then, add 100 µL of each solubilized pellet sample/well in a 96-well clear microplate.

8.4 Measurement:

1. Measure fluorescence of all standards, samples and background controls (Ex/Em 485/535 nm).

9. Data Analysis

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (Standard #1) from all standards, controls and sample readings. This is the corrected fluorescence.
3. If significant, subtract the sample background control from sample readings.
4. Plot the Fluoroscein-5-Thiosemicarbazide (FTC)/FTC Standard Curve.
5. Apply the corrected RFU to the FTC Standard Curve to get B pmol FTC in the sample well.

$$\text{Sample Protein Carbonyl Concentration} = \frac{B * DF * sDF}{V} = \frac{\text{pmol}}{\mu L} = \mu M$$

Where:

B = amount of Protein Carbonyl in the sample well (pmol).

V = sample volume added in the sample wells (100 µL).

DF = Dilution Factor generated by the assay (DF= 10).

sDF = Dilution Factor generated during sample preparation (sDF= 1 if undiluted)

Alternatively, Protein Carbonyl content can be expressed in pmol Carbonyl/mg protein:

$$\text{Carbonyl Content} = \frac{C * 1000}{p} = \frac{\text{pmol}}{\text{mg protein}}$$

Where:

C = sample Protein Carbonyl Concentration (pmol/µl), see equation above

p = sample protein concentration (µg/µl, undiluted).

1000 = conversion factor (1 mg ≡ 1000 µg).

10. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

11. Typical Data

Data provided **for demonstration purposes only.**

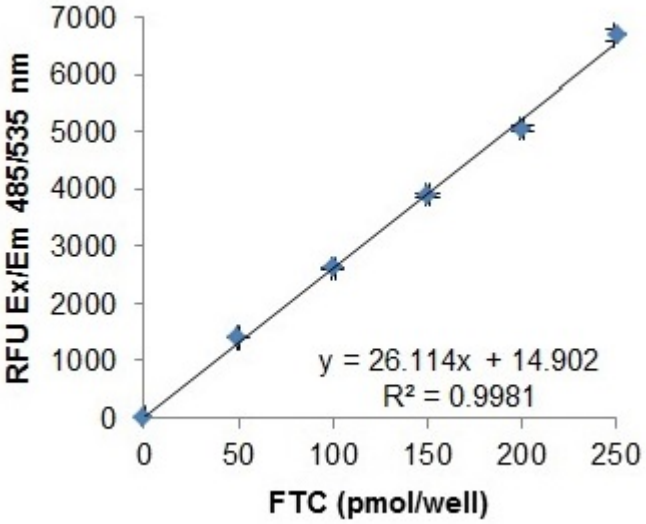

Figure 1. FTC Standard Curve.

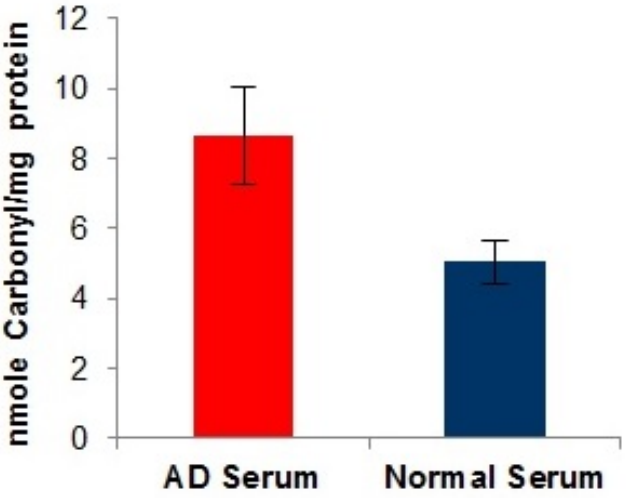

Figure 2. Evaluation of carbonyl content in human serum: 50 µL of human serum from patients with Alzheimer's Disease (AD) or healthy individuals were assayed following kit protocols.

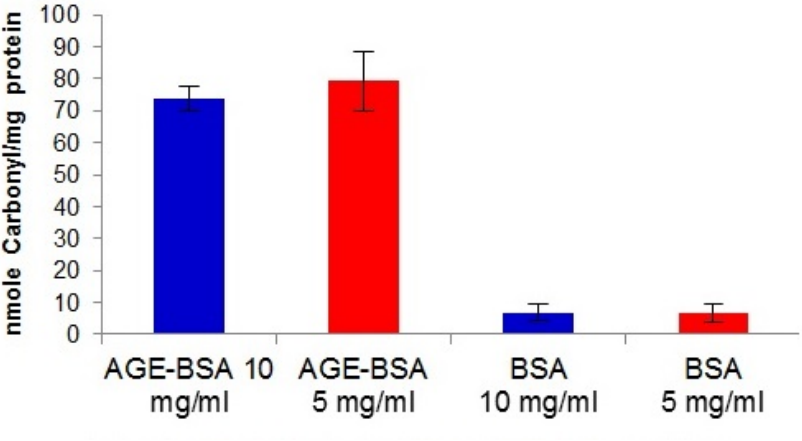

Figure 3. Comparison of AGE-BSA with untreated BSA illustrating the 7-fold increase of carbonyls on AGEs as compared to BSA.

12. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | +86-21-5110-5938 | 400-628-6880
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829