# ab126287

# Protein Carbonyl Content Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Protein Carbonyl content in various samples

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

Version 8a, Last Updated 5 January 2026

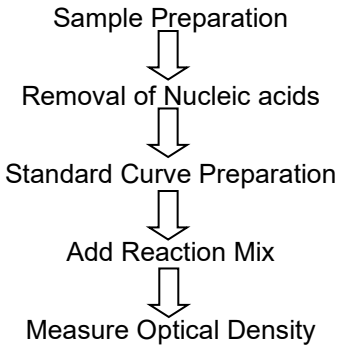
# Table of Contents

# 1. Overview

Protein carbonyl groups are an important and immediate biomarker of oxidative stress. DNPH tagging of protein carbonyls has been one of the most common measures of oxidative stress. DNP hydrazones formed from the reaction are easily quantifiable at 375 nm.

ab126287 Protein Carbonyl Content Assay Kit is designed to provide a simple and accurate method of quantifying carbonyls in protein samples. Using BSA as an example, a 1 mg (~15 nmol) sample has a detection limit of about 0.15 nmol carbonyl, where BSA typically contains approximately 1-3 nmol carbonyl/mg.

# 2. Protocol Summary



# 3. Components and Storage

All components in this kit are shipped on blue ice and are suitable for storage at -20ºC, unless reconstituted. Upon receipt, immediately store kit at -20ºC in the dark. Individual components may be stored at alternative temperatures as show in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

### A. Kit Components

| Item | Quantity | Storage Temperature |
|---|---|---|
| DNPH Solution | 11 mL | -20ºC or +4ºC |
| Trichloroacetic Acid Solution | 3 mL | -20ºC or Ambient |
| Streptozocin Solution | 1 mL | -20ºC |
| Guanidine Solution | 20 mL | -20ºC |
| 96-well clear plate | 1 Unit | -20ºC or +4ºC |

All components are ready to use as supplied but warm to room temperature before use. Keep Trichloroacetic Acid Solution on ice.

We suggest the use 1.5 ml microcentrifuge tubes for all reactions, since they are very convenient for all processing steps.

REAGENTS: Place 10 ml acetone (not provided) in freezer (-20°C) prior to starting the following procedure.

## B. Additional Materials Required

- Microcentrifuge
- Protein Assay Reagents
- Acetone
- Pipettes and pipette tips
- Microplate reader
- 96 well plate
- Orbital shaker

# 4. Assay Protocol

**A. DNPH Assay**

**1. Sample Preparation:**

Homogenise cells using mechanical cell disruption in water only. Common buffer components can interfere with the protein carbonyl content of the sample.

Dissolve samples in $dH_2O$ and centrifuge to spin down any insolubles. Dilute samples with $dH_2O$ to approx. 10 mg/ml protein. If the protein is very dilute, it can be concentrated using a 10 kDa spin filter (ab93349). Use 100 µl of sample containing approximately 0.5-2 mg protein per assay. Include a reagent background control by using 100 µl of $dH_2O$ alone.

**Note:**

Nucleic acids interfere with the assay. Samples containing significant nucleic acid should be treated with Streptozocin (10 µl per 100 µl sample). Leave for 15 min at room temperature, spin at maximum speed for 5 min and transfer supernatant to a new tube. Check 280/260 nm ratio to make sure it is greater than 1.

**2.** Add 100 µl DNPH to each sample, vortex and incubate for 10 min at room temperature.

3. Add 30 µl of Trichloroacetic Acid Solution to each sample, vortex, place on ice for 5 min, spin at maximum speed for 2 min, remove and discard supernatant without disturbing pellet.

4. Add 500 µl of cold acetone to each tube and wash the pellet. 30 seconds in a sonicating bath is typically sufficient to effectively disperse the pellets. Place at -20°C for 5 min then centrifuge for 2 min and carefully remove the acetone.

   **Caution:**
   The acetone pellet is much more easily disturbed than the Trichloroacetic Acid Solution pellet. Repeat the acetone wash step once more to remove free DNPH.

5. Add 200 µl of Guanidine solution and sonicate briefly. Most proteins will be resolubilized easily at this point. If your protein is resistant to resolubilization sonicate for a few seconds then let the solution sit at 60°C for 15-30 min. Spin very briefly to pellet any unsolubilized material and transfer 100 µl of each sample to the 96-well plate.

   **Note:**
   You must use the 96-Well plate included for accurate calculation of carbonyl content.

6. Measure OD at ~375 nm in a microplate reader.

**B. Protein Assay:**

Transfer 5 µl of each sample to another set of wells and perform a protein assay to precisely determine the amount of protein per sample (use BSA as the standard protein when generating your standard curve).

**Notes:**

a) If you are using more than 1 mg protein per sample, it must be diluted so that no more than 25 µg protein is used in the protein assay. Important to correct for any sample losses

b) The BCA Protein Quantification Assay (ab102536) shows minimal interference. The Bradford protein assay is inappropriate for this purpose since guanidine interferes.

# 5. Data Analysis

Correct background by subtracting the value derived from the reagent background control from all readings. The background reading should not be very high but must be subtracted.

Determine protein content of samples from protein standard curve.

**Note:**

The BCA Protein Quantification Assay is best fit by a 2nd order curve rather than a straight line. Determine the carbonyl content as follows:

**C = [(OD 375 nm)/6.364) x (100)] nmol/well**

**CP = nmol carbonyl per mg protein**

**= (C/P) x 1000 x D**

Where:

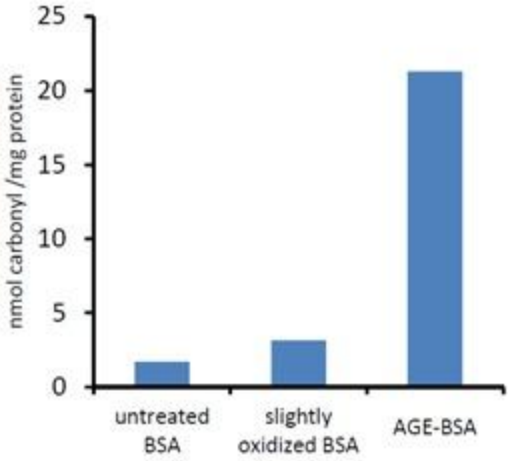
**6.364** is the extinction coefficient using the enclosed 96 well plate in mM (= 22 $mM^{-1}$ $cm^{-1}$ x 0.2893 cm path length in well)

**C** is the Carbonyl in your sample well (nmol)

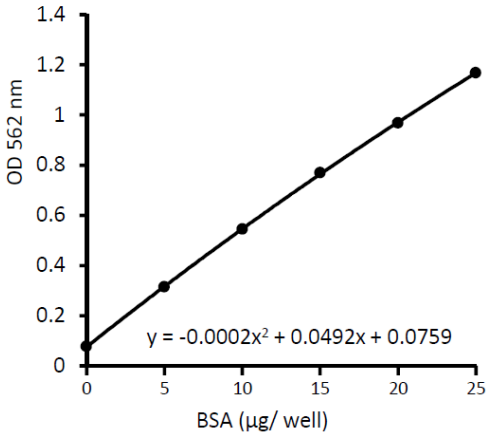
**P** is the protein from standard curve x 20 = µg/well

**D** is the dilution or concentration step applied to sample

**1000** is the factor to convert µg to mg




Representative Data Obtained Using the Protein Carbonyl Content Assay Kit




Typical Standard Curve (from BCA Protein Quantification Assay)

# 6. Troubleshooting

| Problem | Reason | Solution |
|---|---|---|
| Assay not working | Assay buffer at wrong temperature | Assay buffer must not be chilled - needs to be at RT |
| | Protocol step missed | Re-read and follow the protocol exactly |
| | Plate read at incorrect wavelength | Ensure you are using appropriate reader and filter settings (refer to datasheet) |
| | Unsuitable microtiter plate for assay | Fluorescence: Black plates (clear bottoms);<br>Luminescence: White plates;<br>Colorimetry: Clear plates.<br>If critical, datasheet will indicate whether to use flat- or U-shaped wells |
| Unexpected results | Measured at wrong wavelength | Use appropriate reader and filter settings described in datasheet |
| | Samples contain impeding substances | Troubleshoot and also consider deproteinizing samples |
| | Unsuitable sample type | Use recommended samples types as listed on the datasheet |
| | Sample readings are outside linear range | Concentrate/ dilute samples to be in linear range |

| Problem | Reason | Solution |
|---|---|---|
| Samples with inconsistent readings | Unsuitable sample type | Refer to datasheet for details about incompatible samples |
| | Samples prepared in the wrong buffer | Use the assay buffer provided (or refer to datasheet for instructions) |
| | Samples not deproteinized (if indicated on datasheet) | Use the ***10kDa spin column (ab93349)*** |
| | Cell/ tissue samples not sufficiently homogenized | Increase sonication time/ number of strokes with the Dounce homogenizer |
| | Too many freeze-thaw cycles | Aliquot samples to reduce the number of freeze-thaw cycles |
| | Samples contain impeding substances | Troubleshoot and also consider deproteinizing samples |
| | Samples are too old or incorrectly stored | Use freshly made samples and store at recommended temperature until use |
| Lower/ Higher readings in samples and standards | Not fully thawed kit components | Wait for components to thaw completely and gently mix prior use |
| | Out-of-date kit or incorrectly stored reagents | Always check expiry date and store kit components as recommended on the datasheet |
| | Reagents sitting for extended periods on ice | Try to prepare a fresh reaction mix prior to each use |
| | Incorrect incubation time/ temperature | Refer to datasheet for recommended incubation time and/ or temperature |
| | Incorrect amounts used | Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume) |

| Problem | Reason | Solution |
|---|---|---|
| Standard curve is not linear | Not fully thawed kit components | Wait for components to thaw completely and gently mix prior use |
| | Pipetting errors when setting up the standard curve | Try not to pipette too small volumes |
| | Incorrect pipetting when preparing the reaction mix | Always prepare a master mix |
| | Air bubbles in wells | Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates |
| | Concentration of standard stock incorrect | Recheck datasheet for recommended concentrations of standard stocks |
| | Errors in standard curve calculations | Refer to datasheet and re-check the calculations |
| | Use of other reagents than those provided with the kit | Use fresh components from the same kit |

**Technical Support**



**For all technical or commercial enquiries please go to:**

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www.abcam.cn/contactus (China)

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