QuantiChrom™ Carbonyl Assay Kit (DCAR-100)

Quantitative Colorimetric Determination of Carbonyls

DESCRIPTION

CARBONYL groups, such as ketones and aldehydes, are a common indicator of protein oxidation. Protein oxidation is caused as a result of exposure to reactive oxygen species (ROS) and is a common marker in various diseases as well as aging.

BioAssay Systems' Carbonyl Assay Kit is based on an improved method, where 2,4-dinitrophenylhydrazine (DNPH) reacts with carbonyl groups to produce a colored compound at 375 nm. The intensity of this colored compound is directly proportional to the carbonyl groups in the sample.

KEY FEATURES

Fast and sensitive. Use of 100 µL sample. Linear detection range from 12 to 250 µM carbonyl in 96-well plate assay.

Convenient. The procedure involves adding a single working reagent, and reading the absorbance immediately after 30 min.

High-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

Direct Assays: Quantification of carbonyl groups (e.g. ketones, aldehydes) or protein carbonyls in biological samples (e.g. oxidized BSA, etc.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Reagent: 12 mL

Standard: 50 µL (50 mM Carbonyl)

Storage conditions. The kit is shipped at RT. Store all components at -20°C upon receiving. Shelf life: 12 months after receipt.

Precautions: Reagents are for research use only. Briefly centrifuge Standard tube before opening. Equilibrate all components to room temperature prior assay. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

1. *Standards*. Prepare 1 mL of 250 µM Standard by mixing 5 µL of the 50 mM Standard and 995 µL dH_2O. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	250 µM Standard + dH_2O	Carbonyl (µM)
1	500 µL + 0 µL	250
2	300 µL + 200 µL	150
3	150 µL + 350 µL	75
4	0 µL + 500 µL	0

2. Transfer 100 µL standards into separate wells of a clear, flat-bottom 96-well plate. Transfer 100 µL of each sample into separate wells.
3. Add 100 µL Reagent to the four *Standards* and the *Sample Wells.* Tap plate to mix briefly and thoroughly.
4. Incubate 30 min and read optical density at 375 nm (365-385 nm).

CALCULATION

Subtract the blank value (#4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the carbonyl concentration of Sample as follows:

$$[\text{Carbonyl}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

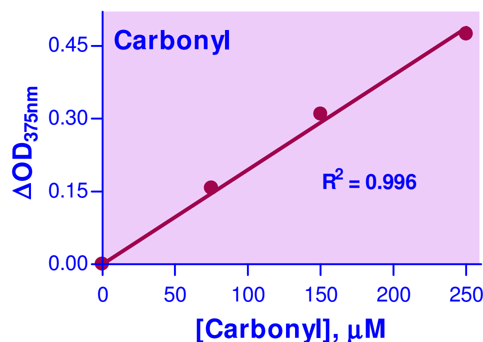
where OD_{SAMPLE} and OD_{BLANK} are optical density readings of the Sample and Blank (Standard #4), respectively. *n* is the sample dilution factor.

Conversions: 10 µM Carbonyl equals 10 nmol/mL.

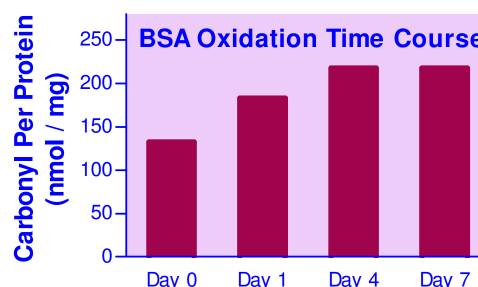
Note: it is recommended to normalize the carbonyl content to protein if measuring carbonyl protein.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), and plate reader.



Carbonyl Standard Curve



BSA Oxidation Time Course

BSA (1 mg/mL) was incubated with 12 mM acetaldehyde at 37°C for 1 week. Carbonyl and protein content were measured. Carbonyl normalized to protein content.

LITERATURE

1. Suzuki, S., et al (2010). Protein Carbonylation. Antioxid. Redox Signal. 12(3): 323-325.
2. Dalle-Donne, I., et al (2003). Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta. 329: 23-38.
3. Weber, D., et al (2015). Determination of protein carbonyls in plasma, cell extracts, tissue homogenates, isolated proteins: Focus on sample preparation and derivatization conditions. Redox Biol. 5: 367-380.

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