Technical Bulletin

Carbonyl Assay Kit

Catalogue Number MAK486

Product Description

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

The 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.

The linear detection range of the kit is 12 – 250 µM. The kit is suitable for the quantification of carbonyl groups (for example, ketones, aldehydes) or protein carbonyls in biological samples (for example, oxidized BSA, etc.).

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Reagent 12 mL

  Catalogue Number MAK486A
- Standard (50 mM Carbonyl) 50 µL

  Catalogue Number MAK486B

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge the Standard vial prior to opening. Equilibrate all components to room temperature prior assay.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

Transfer 100 µL of each Sample into separate wells of a clear 96-well plate.

Standard Curve Preparation

1. Prepare a 250 µM Carbonyl Standard by mixing 5 µL of 50 mM Carbonyl Standard and 995 µL of purified water.
2. Prepare Carbonyl Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.

Preparation of Carbonyl Standards

Well	250 µM Standard	Purified Water	Carbonyl (µM)
1	500 µL	-	250
2	300 µL	200 µL	150
3	150 µL	350 µL	75
4	-	500 µL	-

3. Mix well and transfer 100 µL of each Standard into separate wells of a clear 96-well plate.

Reaction

Transfer 100 µL of Reagent to each Standard and Sample well. Tap plate to mix briefly and thoroughly.

Measurement

1. Incubate the plate for 30 minutes at room temperature.
2. Measure the optical density (OD) at 375 nm.

Results

1. Calculate ΔOD by subtracting the OD reading of the Blank (Standard #4) from the remaining Standard reading values.
2. Plot the ΔOD against Standard concentrations and determine the slope of the standard curve.
3. Calculate the carbonyl concentration of the Sample:

Carbonyl (µM) =

$$\frac{OD_{Sample} - OD_{Blank}}{Slope\ (\mu M^{-1})} \times DF$$

where:

OD_{Sample} = Optical density reading of Sample

OD_{Blank} = Optical density reading of Blank (Standard #4)

DF = Sample dilution factor (DF = 1 for undiluted Samples)

Conversions: 10 µM Carbonyl equals 10 nmol/mL.

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

Figure 1.

Typical Carbonyl Standard Curve

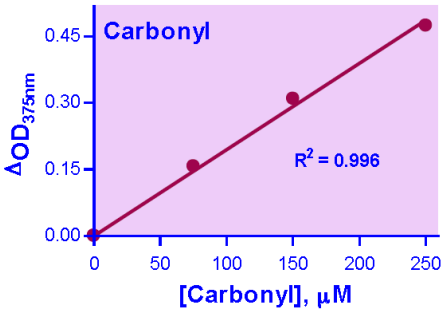
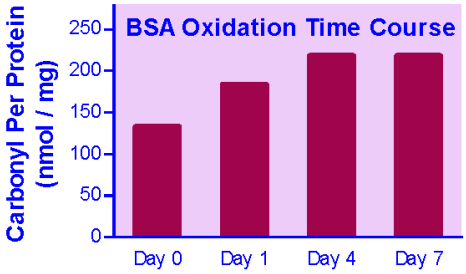


Figure 2.

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 was normalized to protein content.



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