Carbofax 500

Protein Carbonyl Assay Kit

Product Data Sheet

YELEN ANALYTICS

YELEN - Aix-Marseille Université – Service 522, Avenue Escadrille Normandie Niemen, 13013 MARSEILLE

Product Information

Fluorimetric Protein Carbonyl Assay Kit

References: Carbofax 500

Storage Temperature 2 - 8°C except NBDH at -18°C.

Technical Bulletin

Product Description

Oxidative stress can give rise to the formation of protein carbonyl derivatives due to cellular protein oxidation that occurs during pathological processes. Carbonylation of proteins is characterized by the introduction of carbonyl groups in protein structure, and the quantification of the carbonyl content of cell proteins is a useful indicator of oxidative damage and a frequently studied oxidation target product.

The Carbofax Fluorimetric Protein Carbonyl Assay Kit provides a simple and direct procedure for measuring carbonyl content in proteins and biological samples. Carbonyl content is determined by the derivatization of protein carbonyl groups with 7-hydrazino-4-nitrobenzo-2,1,3-oxadiazole (NBD-H) leading to the formation of fluorescent adducts, which can be detected by fluorescence (Excitation 480 / Emission 560), proportional to the carbonyls present.

The limit of detection for this kit will vary depending upon the nature of the protein being tested. With bovine serum albumin (BSA), this kit can detect carbonyl levels of 0,15 nmole of carbonyls/mg of BSA. Note that BSAox (Oxidized Bovine Serum Albumin) is not provided in this kit.

Components

The kit is sufficient for 500 assays in 96-well plates.

2 Lyophilised NBDH mix powder in 1,5 ml bottle: ……. storage at -18°C.

1 Reconstitution solution (1,5 ml): …… storage at 2 to 8°C.

1 Carbofax Buffer solution (12 ml): …… ……. storage at 2 to 8°C.

Reagents and Equipment Required but Not Provided

Fluorimeter multiwell plate reader capable of reading at Ex: 480 nm and Em: 562 nm, 10% Streptozocin solution, 30% TCA solution and Kit for protein determination.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses.

Preparation Instructions

1. **Carbofax Buffer** and **Reconstitution solution** should be placed at room temperature 15 min before experiment.
2. Add 500 µl of **Reconstitution solution** in **1 Lyophilised NBDH mix powder** bottle**,** sonicate (1 min) and vortex vigorously (30 sec) (after use, this solution can be stored at -18°C).
3. Add 30 µl of this **reconstituted NBDH solution** per ml of **Carbofax Buffer** (100 µl of Carbofax buffer per assay).

Procedures

All samples and standards should be run in duplicate or triplicate.

Sample Preparation

Samples can be prepared in any suitable aqueous carbonyl-free buffer and centrifuged to remove any insoluble material.

Add 100 µl of experiment aqueous carbonyl-free buffer into a well to serve as a reagent background control.

Note: Nucleic acids may interfere with the assay. If samples contain significant amounts of nucleic acids, treat samples with 10 µl of 10% Streptozocin solution per 100 µl of sample. Incubate at room temperature for 15 min, centrifuge at 13,000*g* for 5 minutes and then, transfer supernatant to a new tube.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

Fluorescent Assay Reaction

1. Add 100 µl of sample to each well

2. Add 100 µl of **reconstituted NBDH solution** to each sample well

3. After 15 min at 37°C, measure fluorescence at Ex: 480 nm and Em: 562 nm.

Note: it possible to substrate fluorescence due to free carbonyls content by pre-treatment of sample: precipitate proteins by the addition of 2 vol of 30% TCA. Following 10 min of ice incubation, centrifuge at 3000*g* for 10 min at 4°C. Then, measure 100 µl of supernatants free carbonyl fluorescence by following instructions above.

Carbonyl Content

It is possible to quantify carbonyls content with internal standard or standard curve of BSAox or any other oxidized protein.

Reference : Stocker *et al.*, *Analytical Biochemistry* Vol. 482, 1 August 2015, Pages 55-56.

Contact information

Further information on YELEN products, including product bibliographies, is available from our website. Please visit our website — **http://yelen-analytics.com** — for the most up-to-date information.

YELEN products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Limited Use Label License

For research use only. Not intended for any animal or human therapeutic or diagnostic use. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. YELEN will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, YELEN is willing to accept return of the product with a full refund.