Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
info@listarfish.it
www.listarfish.it

Manual

Carbonyl protein ELISA

For the in vitro determination of protein-bound carbonyls in human serum and plasma

Valid from 2019-03-26

REF **K 7870**

Σ 96

+8°C
+2°C

IVD

CE

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 70190-363
e.mail: info@immundiagnostik.com www.immundiagnostik.com

Table of Contents

1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of protein carbonyls in human serum and plasma. For *in vitro* diagnostic use only.

2. INTRODUCTION

Reactive oxygen species (ROS) can oxidise proteins, lipids, and DNA, causing damage of their structure and function as well as cell injury. Proteins are oxidised by free radicals, whereby the constituent amino acids are variously modified or degraded. The modifications result in new functional groups such as carbonyl or hydroxyl groups, which may lead to protein fragmentation, formation of protein-protein cross-linkages, disruption of the tertiary structure and loss of functional activity. In addition, ROS are directly associated with diseases like atherosclerosis, rheumatoid arthritis, Alzheimer's and Parkinson's disease as well as ageing and cancerogenesis.

Protein carbonyls are formed by a variety of oxidative mechanisms and are sensitive indices of oxidative injury. The quantity of protein carbonyls in a protein sample can be determined by derivatising with dinitrophenyl-hydrazine (DNPH) and measuring the bound anti-DNPH antibodies. The ELISA method enables carbonyls to be measured quantitatively with microgram quantities of protein.

Indications

- Atherosclerosis
- Alzheimer's disease
- Parkinson's disease
- Rheumatoid arthritis
- Uremia
- Diabetes
- Ageing
- Cancerogenesis

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 7870	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	1 x 100 ml
K 7870	STD	Standards, lyophilised (see specification for concentrations)	4 x 5 vials

Cat. No.	Label	Kit components	Quantity
K 7870	CTRL 1	Control, lyophilised (see specification for range)	4 x 1 vial
K 7870	CTRL 2	Control, lyophilised (see specification for range)	4 x 1 vial
K 7870	CONJ	Conjugate concentrate, peroxidase-labelled	1 x 200 µl
K 7870	CONJBUF	Conjugate dilution buffer, ready-to-use	1 x 15 ml
K 7870	AB	Detection antibody concentrate, (secondary antibody)	1 x 200 µl
K 7870	ABBUF	Antibody dilution buffer, ready-to-use	1 x 15 ml
K 7870	DER	Derivatization reagent	2 x 5 ml
K 7870	ASYBUF	Assay buffer, ready-to-use	2 x 100 ml
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Multi-channel pipets or repeater pipets
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. PREPARATION AND STORAGE OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- The **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. **Reconstitution** details are given in the **specification data sheet**. **Standards and controls** (reconstituted STD and CTRL) **are not stable and cannot be stored.**
- The **DER** (derivatisation reagent) is prepared as a saturated solution. Crystals can occur due to the high salt concentration. The DER (derivatisation reagent) is used as such, without removing the crystals.
- **Preparation of the detection antibody**: The **detection antibody concentrate (AB)** has to be diluted **1:101** in **antibody dilution buffer (ABBUF)** (100 µl AB + 10 ml ABBUF). The **AB** is stable at **2–8 °C** until the expiry date stated on the label. **Detection antibody** (1:101 diluted AB) **is not stable and cannot be stored.**
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in **conjugate dilution buffer (CONJBUF)** (100 µl CONJ + 10 ml CONJBUF). The **CONJ** is stable at **2–8 °C** until the expiry date stated on the label. **Conjugate** (1:101 diluted CONJ) **is not stable and cannot be stored.**
- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label) when stored at **2**–**8 °C**.

6. SPECIMEN COLLECTION AND PREPARATION

Serum and plasma samples are suited for this test system.

Storage

Samples should be sent cooled; they are stable for 24 h at room temperature.

Derivatisation of samples

1.	Label a tube for each **sample**.
2.	Add **25 µl sample** into each tube
3.	Add **100 µl derivatisation reagent** (DER) into each tube.
4.	Close the tubes and vortex the content well.
5.	For derivatisation, incubate for **30 min at 37 °C*.**

* Alternatively, incubate over night at 4 °C.

Sample dilution

The derivatised samples must be diluted **1:20 000 in assay buffer** before use in the test:

- **30 µl** derivatised sample + **570 µl** assay buffer, mix well
  = **1:20** (**dilution I**)
- **30 µl** dilution II + **570 µl** assay buffer, mix well
  = **1:20** (**dilution II**)
- **20 µl** dilution II + **980 µl** assay buffer, mix well
  = **1:50** (**dilution III**).
  This results in a final dilution of 1:20 000.

For analysis, pipet **100 µl** of **dilution III** per well.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of protein-bound carbonyls.

Assay standards, controls and patient samples are derivatised and added into the wells of precoated microplate. The quantification of the bound proteins is performed by adding of second antibody which is biotinylated and detected by peroxidase la-

belled streptavidin. Tetramethylbenzidin (TMB) is used as a peroxidase substrate. The intensity of the colour is directly proportional to the concentration of carbonyl proteins. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. Carbonyl proteins in the patient samples are determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	**Before use**, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each **100 µl standards/controls/prepared samples** (dilution III) into the respective wells.
3.	Cover plate tightly and incubate for **1 hour at 37 °C**.
4.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add **100 µl detection antibody** (AB) into each well.
6.	Cover the plate tightly and incubate for **1 hour at 37 °C.**
7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add **100 µl conjugate** (diluted CONJ) into each well.
9.	Cover the plate tightly and incubate for **1 hour at 37 °C.**

10.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
11.	Add **100 µl substrate** (SUB) into each well.
12.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.
13.	Add **100 µl stop solution** (STOP) into each well and mix well using the shake mode of the microtiter plate reader.
14.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm as a reference.

* The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the "4 parameter algorithm".

1. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e. g. 0.001).

2. Point-to-point calculation

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. Spline algorithm

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Plasma and serum samples

Since the sample dilution is already considered in the calibration curve, the dilution factor is 1.

9. LIMITATIONS

Samples with an OD higher than the OD of the highest standard can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

Based on Immundiagnostik AG studies of matrix samples of apparently healthy persons (n = 41), a reference rage of 70–200 U/ml was estimated.

11. PERFORMANCE CHARACTERISTICS

Spiking Recovery

Sample	Unspiked Sample [U/ml]	Spike [U/ml]	expected [U/ml]	measured [U/ml]
A	66.5	33.0	99.5	90.7
	66.5	90.0	156.5	148.6
	66.5	280.0	346.5	340.6
B	140.4	33.0	173.4	171.6
	140.4	90.0	230.4	243.0
	140.4	280.0	420.4	400.0

Sample	Unspiked Sample [U/ml]	Spike [U/ml]	expected [U/ml]	measured [U/ml]
C	116.8	33.0	149.8	130.5
	116.8	90.0	206.8	168.2
	116.8	280.0	396.8	375.2

Precision and reproducibility

Intra-Assay (n = 26)

Sample	Carbonyl proteins mean value [U/ml]	Standard deviation (SD) [%]
1	280.9	6.5
2	553.4	5.2

Inter-Assay (n = 14)

Sample	Carbonyl proteins mean value [U/ml]	Standard deviation (SD) [%]
1	77.9	12.5
2	170.2	6.2
3	127.7	7.9

12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Control samples should be analysed with each run.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or Proclin as bactericides. Sodium azide and Proclin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.

- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

1. Beal, M. F. Oxidatively modified proteins in aging and disease. *Free radical biology & medicine* **32**, 797–803 (2002).
2. Berlett, B. S. & Stadtman, E. R. Protein oxidation in aging, disease, and oxidative stress. *The Journal of biological chemistry* **272**, 20313–6 (1997).
3. Buss, H., Chan, T. P., Sluis, K. B., Domigan, N. M. & Winterbourn, C. C. Protein carbonyl measurement by a sensitive ELISA method. *Free radical biology & medicine* **23**, 361–6 (1997).
4. Cao, G. & Cutler, R. G. Protein oxidation and aging. I. Difficulties in measuring reactive protein carbonyls in tissues using 2,4-dinitrophenylhydrazine. *Archives of biochemistry and biophysics* **320**, 106–14 (1995).
5. Davies, K. J., Delsignore, M. E. & Lin, S. W. Protein damage and degradation by oxygen radicals. II. Modification of amino acids. *The Journal of biological chemistry* **262**, 9902–7 (1987).
6. Dean, R. T., Fu, S., Stocker, R. & Davies, M. J. Biochemistry and pathology of radical-mediated protein oxidation. *The Biochemical journal* **324** (Pt 1, 1–18 (1997).
7. Descamps-Latscha, B. & Witko-Sarsat, V. Importance of oxidatively modified proteins in chronic renal failure. *Kidney international.* **Supplement 78**, S108–13 (2001).
8. Galli, F. Protein damage and inflammation in uraemia and dialysis patients. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **22** Suppl 5, v20–36 (2007).
9. Gladstone, I. M. & Levine, R. L. Oxidation of proteins in neonatal lungs. *Pediatrics* **93**, 764–8 (1994).
10. Lenz, a G. et al. Oxidatively modified proteins in bronchoalveolar lavage fluid of patients with ARDS and patients at-risk for ARDS. *The European respiratory journal* **13**, 169–74 (1999).
11. Levine, R. L. Carbonyl modified proteins in cellular regulation, aging, and disease. *Free radical biology & medicine* **32**, 790–6 (2002).
12. Levine, R. L. & Stadtman, E. R. Oxidative modification of proteins during aging. *Experimental gerontology* **36**, 1495–502 (2001).
13. Marnett, L. J., Riggins, J. N. & West, J. D. Endogenous generation of reactive oxidants and electrophiles and their reactions with DNA and protein. *The Journal of clinical investigation* **111**, 583–93 (2003).

14. Matzi, V. et al. The impact of preoperative micronutrient supplementation in lung surgery. A prospective randomized trial of oral supplementation of combined alpha-ketoglutaric acid and 5-hydroxymethylfurfural. *European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery* **32**, 776–82 (2007).

15. Reznick, A. Z. et al. Modification of plasma proteins by cigarette smoke as measured by protein carbonyl formation. *The Biochemical journal* **286** (Pt 2, 607–11 (1992).

16. Smith, C. D. et al. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 10540–3 (1991).

17. Stadtman, E. R. & Oliver, C. N. Metal-catalyzed oxidation of proteins. Physiological consequences. *The Journal of biological chemistry* **266**, 2005–8 (1991).

18. Starke-Reed, P. E. & Oliver, C. N. Protein oxidation and proteolysis during aging and oxidative stress. *Archives of biochemistry and biophysics* **275**, 559–67 (1989).

19. Wiseman, H. & Halliwell, B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *The Biochemical journal* **313** (Pt 1, 17–29 (1996).

20.

Used symbols:


Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet