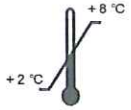
Manual

Carbonyl Protein ELISA Kit

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

Valid from 25.10.2012

REF K 7870



RUO

1. Intended use

This ELISA Kit is intended for the determination of protein carbonyls in human serum and plasma. For research use only.

2. Introduction

Reactive oxygen species (ROS) can oxidize proteins, lipids, and DNA, causing damage of their structure and function as well as cell injury. Proteins are oxidized 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 derivatizing 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.

Indication

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

3. Principle of the test

Assay standards, controls and patient samples are derivatized and added into the wells of precoated microplate. The quantification of the bound proteins is performed by adding of a 2. antibody which is biotinylated and detected by peroxidase labeled streptavidin. Tetramethylbenzidin (TMB) is used as a peroxidase substrate. The intensity of the color 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..

4. Material supplied

Cat. No	Content	Kit Components	Quantity
K7870MTP	PLATE	One holder with strips	12 x 8 wells
K7870WP	WASHBUF	Wash buffer concentrate (10 fold)	1 x 100 ml
K7870ST	STD	Standard stock solution, lyophilized	1 x 1 vial
K7870KO1	CTRL	Control, lyophilized	1 x 1 vial
K7870KO2	CTRL	Control, lyophilized	1 x 1 vial
K7870K	CONJ	Conjugate, peroxidase-labeled	1 x 200 µl
K7870KV	CONJBUF	Conjugate dilution buffer	1 x 15 ml
K7870A2	AB	1. Antibody	1 x 200 µl
K7870VP	ABBUF	Antibody dilution buffer	1 x 15 ml
K7870DR	DER	Derivatization reagent	1 x 5 ml
K7870AP	ASYBUF	Assay buffer	1 x 100 ml
K7870TMB	SUB	TMB substrate	1 x 15 ml
K7870AC	STOP	Stop solution	1 x 15 ml

5. Material Required but not supplied

- Ultra pure water*
- Precision pipettors and disposable tips to deliver 0.5 - 1000 µl
- Foil to cover the microtiter plate
- A multi-channel dispenser or repeating dispenser for washing
- Vortex-Mixer
- Disposable standard laboratory plastic tubes
- Microtiter plate reader at 450

*Immundiagnostik AG recommends the use of Ultra Pure 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 0,055µS/cm at 25°C (≤18,2MΩ cm).

6. Preparation and storage of reagents

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay.** The kit can be used up to 4 times within the expiry date stated on the label.
- The **ELISA WASHBUF** (wash buffer concentrate) must be diluted with ultra pure water **1:10** before use (100 ml WASHBUF + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at 37°C in a water bath before dilution. The **buffer concentrate** is stable at **2–8°C** until the expiry date stated on the label. Diluted **buffer solution (wash buffer)** can be stored in a closed flask at **2–8°C for one month.**
- The lyophilized **STD** (standard) and **CTRL** (controls) are stable at **2-8°C** until the expiry date stated on the label. The **STD** (standard) and **CTRL** (controls) must be reconstituted as stated in the product specification. **Reconstituted standard and controls are stable for one week at 2-8°C.**
- The **DER** (Derivatization reagent) is prepared as a saturated solution. Crystals can occur due to the high salt concentration. The **DER** (Derivatization reagent) is used as such, without removing the crystals.

- The **AB** (2. Antibody) must be diluted **1:100 in ABBUF** (antibody dilution buffer): e.g. 100 µl AB (2. Antibody) + 10 ml ABBUF. The undiluted 2. Antibody is stable at **2-8 °C** until the expiry date stated on the label.
- The **CONJ** (conjugate) must be diluted **1:100** in **CONJBUF** (conjugate dilution buffer) (100 µl CONJ + 10 ml CONJBUF). The undiluted conjugate is stable at **2-8 °C** until the expiry date stated on the label.
- All other test reagents can be stored at 2-8° C and are stable until the expiry date (see label of test package).

7. PRECAUTIONS

- Stop as well as derivatization solution is composed of strong acid. Even diluted, they still must be handled with care. They can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
- Reagents should not be used beyond the expiration date shown on kit label.

8. SAMPLE AND TEST PREPARATION

- Serum and plasma samples are suited for this test system.
- Samples should be sent cooled; they are stable for 24 h at room temperature.

9. ASSAY PROCEDURE

Procedural notes

- Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure, that are not coordinated with the producer, may influence the test results. Immundiagnostik can therefore not be held reliable for any damage resulting from this.
- The assay should always be performed according to the enclosed manual.

Sample preparation and test procedure

Derivatization

1.	Label a tube for each of **STD** (standard), **CTRL** (control) or **SAMPLE** (sample)
2.	Add in each tube **10µl** of **STD** (standard), **CTRL** (control) or **SAMPLE** (sample)
3.	Add in each tube **40µl** of **DER** (derivatization reagent)
4.	Close the tubes and vortex-mix well the content
5.	Allow the derivatization to proceed for **30 min** at 37°C

Dilution of Standard and Controls

Dilution I

1:40 Dilution

10 µl of each, derivatized **STD** (standard) and **CTRL** (control) + **390 µl ASYBUF** (Assay buffer)

Dilution II

1:100 Dilution

10 µL Dilution I + 990 µl ASYBUF (Assay buffer)

Standard dilution series

Dilution II of the derivatized standard corresponds to S5 (standard 5).

S4= 200 µL S5 + 400 µL ASYBUF (Assay buffer)

S3= 200 µL S4 + 400 µL ASYBUF (Assay buffer)

S2= 200 µL S3 + 400 µL ASYBUF (Assay buffer)

S1= 500 µL ASYBUF (Assay buffer)

100 µl of each, diluted standards and dilution II of the controls, are used in the test.

Dilution of Samples

The derivatized samples must be diluted **1:20000** with **ASYBUF** (Assay buffer) before use in the test:

Dilution I

5 µl of derivatized sample + **995 µl ASYBUF** (Assay buffer)

Dilution II

10 µl of Dilution I + 990 µl ASYBUF (Assay buffer)

100 µl of Dilution II of each sample per well are used in the test.

Test procedure ELISA

1. Take as many microtiter strips (PLATE) as needed from kit. Store unused strips in the closed original package bag at 2-8°C. Strips are stable until the expiry date stated on the label
2. Wash the coated microtiter plate **5 times** with **250 µl** of **diluted wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution
3. For the analysis in duplicate, pipette **2 x 100 µl** of **STD** (standards), **CTRL** (control) and **SAMPLE** (samples) **from dilution II** into the respective well of the microtiter plate
4. Cover plate tightly and incubate for **1 hour at 37°C**
5. Aspirate the contents of each well. Wash **5 times** by dispensing **250 µl** of **diluted wash buffer** into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution
6. Add **100 µl** of **diluted 2.antibody** into each well
7. Cover the plate tightly and incubate for **1 hour at 37°C**
8. Aspirate the contents of each well. Wash **5 times** by dispensing **250 µl** of **diluted wash buffer** into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution

9. Add **100 µl of diluted conjugate** into each well
10. Cover the plate tightly and incubate for **1 hour at 37°C**
11. Aspirate the contents of each well. Wash **5 times** by dispensing **250 µl** of **diluted wash buffer** into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution
12. Add **100 µl** of **SUB** (TMB substrate) into each well
13. Incubate for **10-20 min at room temperature** in the dark*
14. Add **100 µl** of **STOP** (stop solution) into each well, mix thoroughly
15. Determine **absorption** immediately with an ELISA reader at **450 nm**. If the highest extinction of the standards **(STD)** is above the range of the photometer, absorption must be measured immediately at **405 nm** and the obtained results used for evaluation. If possible, the extinctions from each measurement should be compared with extinctions obtained at a reference wavelength, e. g. 595 nm, 620 nm, 630 nm, 650 nm and 690 nm can be used

*The intensity of the color change is temperature sensitive. We recommend to observe the color change and to stop the reaction upon good differentiation.

10. Evaluation of Results

A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from standard. The concentration of patient samples is determined directly from the linear standard curve.

A 4-parameter curve fitting equation is recommended for evaluation of the results.

Expected values

Normal range

follows

11. PERFORMANCE CHARACTERISTICS

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=11)		
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

Recovery

n = 3

Sample [U/ml]	Spike [U/ml]	Carbonyl proteins expected (U/ml)	Carbonyl proteins measured (U/ml)
66,5	33,0	99,5	90,7
66,5	90,0	156,5	148,6
66,5	280,0	346,5	340,6
140,4	33,0	173,4	171,6
140,4	90,0	230,4	243,0
140,4	280,0	420,4	400,0
116,8	33,0	149,8	130,5
116,8	90,0	206,8	168,2
116,8	280,0	396,8	375,2

12. REFERENCES

Beal MF (2002) Oxidatively modified proteins in aging and disease. *Free Radical Biology and Medicine* **32(9):** 797-803

Berlett BS and Stadtman ER (1997) Protein oxidation in aging, disease, and oxidative stress. *J Biol. Chem.* **272:** 33-20316

Buss H, Chan TP, Sluis KB, Domigan NM and Winterbourn CC (1997) Protein carbonyl measurement by a sensitive ELISA method. *Free Radic Biol Med* **23:** 361-366

Cao G and Cutler RG (1995) Protein oxidation and aging. I. Difficulties in measuring reactive protein carbonyls in tissues using 2,4-dinitrophenylhydrazine. *Arch. Biochem. Biophys.* **320:** 106–114

Davies KJA and Delsignore ME (1987) Protein damage and degradation by oxygen radicals. III. Modification of secondary and tertiary structure. *J. Biol. Chem.* **262:** 9908–9913

Dean RT, Fu S, Stocker R and Davies MJ (1997) Biochemistry and pathology of radical-mediated protein oxidation. *Biochem. J.* May 15; **324**: 1-18

Descamps-Latscha B, Drueke T, Witko-Sarsat V (2001) Dialysis-induced oxidative stress: biological aspects, clinical consequences, and therapy. *Semin Dial.* May-Jun;**14(3):** 193-9

Galli F (2007) Protein damage and inflammation in uraemia and dialysis patients. *Nephrol Dial Transplant.* Jul; 22 Suppl **5:** v20-v36. Review

Gladstone IMJ and Levine RL (1994) Oxidation of proteins in neonatal lungs. *Pediatrics* **93:** 764–768

Lenz A-G, Jorens PG, Meyer B, De Backer W, Van Overveld F, Bossaert L and Maier KL (1999) Oxidatively modified proteins in bronchoalveolar lavage fluid of patients with ARDS and patients at-risk for ARDS. *Eur Respir J.* **13:** 169-174

Levine RL (2002) Carbonyl modified proteins in cellular regulation, aging, and disease. *Free Radic Biol Med.* May 1;**32(9):** 790-6. Review

Levine RL and Stadtman ER (2001) Oxidative modification of proteins during aging. *Exp Gerontol.* Sep; **36(9):** 1495-502. Review

Marnett LJ, Riggins JN, West JD (2003) Endogenous generation of reactive oxidants and elektrophiles and their reactions with DNA and protein. *J. Clin. Invest.* **111:** 583–593

Matzi V, Lindenmann J, Muench A, Greilberger J, Juan H, Wintersteiger R, Maier A, Smolle-Juettner FM (2007) The impact of preoperative micronutrient supplementation in lung surgery. A prospective randomized trial of oral supplementation of combined a-ketoglutaric acid and 5-hydroxymethylfurfural. *European Journal of Cardio-thoracic Surgery* **32:** 776-782

Reznick AZ, Cross CE, Hu ML, Suzuki YJ, Khwaja S, Safadi A, Motchnik PA, Packer L, Halliwell B (1992) Modification of plasma proteins by cigarette smoke as measured by protein carbonyl formation. *Biochem. J.* **286:** 607–611

Smith CD, Carney JM, Starke-Reed PE, Oliver CN, Stadtman ER, Floyd RA, Markesbery WR (1991) Excess brain protein oxidation and enzyme dysfunction in normal, aging and in Alzheimer disease. *Proc. Natl. Acad. Sci. USA* **88:** 10540–10543

Stadtman ER and Oliver CN (1991) Metal-catalyzed oxidation of proteins. Physiological consequences. *J. Biol. Chem.* **266:** 2005–2008

Starke-Reed PE and Oliver CN (1989) Protein oxidation and proteolysis during aging and oxidative stress. *Arch. Biochem. Biophys.* **275:** 559–567

Wiseman H and Halliwell B (1996) Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem. J.* **313:** 17–29

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- Test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the test package are for research use only.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, do not assemble cavities of different microplates for analyses, even if the microplates are of the same charge.
- Guidelines for medical laboratories should be observed.
- The assay should always be performed according the enclosed manual.
- 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 wrong use.

Used symbols:

	Temperature limitation	REF	Catalogue Number
IVD	In Vitro Diagnostic Medical Device		Contains sufficient for <n> tests
	Manufacturer		Use by
LOT	Lot number	RUO	For research use only