Verwendete Symbole:

	Temperaturbegrenzung	REF	Bestellnummer
IVD	In-Vitro-Diagnostikum		Inhalt ausreichend für <n> Prüfungen
	Hersteller		Verwendbar bis
LOT	Chargenbezeichnung	RUO	Nur für Forschungszwecke

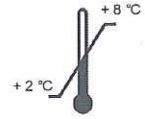
Manual

Carbonyl Protein ELISA Kit

For the determination of protein carbonyls in biological samples

Valid from 05.12.2013

REF **K 7822**



RUO

1. INTENDED USE

This ELISA Kit is intended for the determination of protein carbonyls in biological samples such as EDTA-plasma, bronchoalveolar lavage fluid and cerebrospinal fluid, cell extracts and other soluble protein samples. 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

Samples containing protein are reacted with DNPH; then the non-protein constituents and unconjugated DNPH are separated by ultracentrifugation. The proteins are adsorbed to an ELISA plate and incubated with anti-DNPH antibody followed by antibody-linked horseradish peroxidase. Absorbances are related to a standard curve prepared with oxidized serum albumin.

The carbonyl protein content is calculated from the estimated carbonyl concentration and the total protein content of the sample. **For this reason, a parallel determination of the protein content is required.**

4. MATERIAL SUPPLIED

Cat. No	Content	Kit Components	Quantity
K 7822MTP	PLATE	One holder with strips	12 x 8 wells
K 7822WP	WASHBUF	Wash buffer concentrate (10 fold)	1 x 100 ml
K 7822ST	STD	Standard stock solution	1 x 50 µl
K 7822KO	CTRL	Control	1 x 50 µl
K 7822K	CONJ	Conjugate, peroxidase-labeled	1 x 22 ml
K 7822A1	AB	1. Antibody	1 x 240 µl
K 7822PV	ABBUF	Antibody dilution buffer	1 x 30 ml
K 7822DR	DER	Derivatization reagent	1 x 9 ml
K 7822AP	ASYBUF	Assay buffer	2 x 100 ml
K 7822TMB	SUB	TMB substrate	2 x 15 ml
K 7822AC	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
- Centrifuge capable of 11000 x g
- Vortex-Mixer
- Standard laboratory **reaction vessels (cups) made of polypropylene**
- Centrifugal filtration concentrators can be ordered from Immundiagnostik (Cat. No K 7822ZR)
- Protein quantification test can be ordered from Immundiagnostik (Cat. No K 7822BCA)
- Microtiter plate reader at 450 nm (reference wave length 620 or 690 nm)

* 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 0.055 µS/cm at 25 °C (≥ 18.2 MΩ 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 **WASHBUF** (wash buffer concentrate) should 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 room temperature or at 37°C using a water bath before dilution of the buffer solutions. The **buffer concentrate** is stable at **2-8°C** until the expiry date stated on the label. **Diluted buffer solution** can be stored in a closed flask at **2-8°C for one month.**

- 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** (1. Antibody) must be diluted **1:101 in ABBUF** (Antibody dilution buffer): e.g. Preparation of reagents for 1 plate:

  220 µl AB (1. Antibody) + 22 ml ABBUF (Antibody dilution buffer)

  Diluted AB-solution can be stored **for 2 days at 2-8°C** in a closed flask.

- 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

- Plasma, bronchoalveolar lavage fluid and cerebrospinal fluid, cell extracts and other soluble protein 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

- The carbonyl protein content is calculated from the estimated carbonyl concentration and the total protein content of the sample. **For this reason, a parallel determination of the protein content is required.**
- 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. Bring all reagents and samples to room temperature (18-26°C)
2. Label the centrifugal filtration concentrators for **STD** (standard), **CTRL** (control), **ASYBUF** (blank) and **SAMPLE** (samples) and place them in the collecting vials
3. Add in each centrifugal filtration concentrator **80µl** of **DER** (derivatization reagent)
4. Add **4 µl** of each **STD** (standard), **CTRL** (control), **ASYBUF** (blank) and **SAMPLE** (sample) in the corresponding centrifugal filtration concentrator containing the derivatization reagent. Mix by repeated pipetting of the mixture up and down and close the centrifugal filtration concentrator
5. Allow the derivatization to proceed for **45 min** at room temperature
6. Centrifuge all centrifugal filtration concentrators at **11000 x g** for **15 min**
7. **Add 60 µl of ASYBUF** (assay buffer) in all centrifugal filtration concentrators
8. **Repeat step 6 and 7 four times**

Dilution I

1:4 Dilution

- 180 µl ASYBUF (assay buffer) + 60 µl **Sample** supernatant after derivatization
- 180 µl ASYBUF (assay buffer) + 60 µl **Control** supernatant after derivatization
- 180 µl ASYBUF (assay buffer) + 60 µl **Blank** supernatant after derivatization (S1)
- 180 µl ASYBUF (assay buffer) + 60 µl **Standard** supernatant after derivatization (S6); prepare a dilution series

Standard dilution series

S5= 100 µL S6 + 100 µL ASYBUF (assay buffer)
S4= 100 µL S5 + 100 µL ASYBUF (assay buffer)
S3= 100 µL S4 + 100 µL ASYBUF (assay buffer)
S2= 100 µL S3 + 100 µL ASYBUF (assay buffer)

Dilution II

1:20 Dilution

- 40 µL Dilution I + 760 µl ASYBUF (assay buffer)

This dilution is used for protein determination of standard 6 (S6), control and the respective samples. We recommend incubating the protein determination test (BCA-Test) at 37°C for 3 hours.

Dilution III

1:100 Dilution

- 10 µL Dilution II + 990 µl ASYBUF (assay buffer)

This dilution is used for the ELISA 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. For the analysis in duplicate, pipette **2 x 200 µl** of **STD** (standards), **CTRL** (control), **BLANK** (blank) and **SAMPLE** (samples) **from dilution III** into the respective well of the microtiter plate
3. Cover plate tightly and incubate for **3 hours at 37°C or over night at 2-8°C**
4. 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
5. Add **200 µl** of **diluted AB** (anti-DNPH-antibody) into each well

6. Cover the plate tightly and incubate for **20 min at room temperature** (18-26°C). Important: Do not shake!
7. 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
8. Add **200 µl** of **CONJ** (conjugate, goat-anti-rabbit-peroxidase-labeled) into each well
9. Cover the plate tightly and incubate for **20 min at room temperature** (18-26°C). Important: Do not shake!
10. 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
11. Add **200 µl** of **SUB** (TMB substrate) into each well
12. Incubate for **15-20 min at room temperature** in the dark*
13. Add **50 µl** of **STOP** (stop solution) into each well, mix thoroughly
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 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.

The protein carbonyl content is calculated according to the following formula:

$$CP_{Sample}\ [pmol/mg]\ standardized = \frac{CP_{Sample}\ [pmol/mg] \times Proteins_{Standard}\ [mg/ml]}{Proteins_{Sample}\ [mg/ml]}$$

CP_{Sample} : Carbonyl protein content of the sample in pmol/mg, estimated from the standard curve in the assay

$Proteins_{Standard}$: Protein content of dilution II of the highest standard (S6), estimated with the BCA-Test in mg/ml

$Proteins_{Sample}$: Protein content of the sample dilution II, estimated with the BCA-Test in mg/ml

Expected values

Normal range

EDTA-plasma	75 – 200 pmol/mg

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n=4)		
Probe	Carbonyl proteins [pmol/mg]	Standard Deviation (SD) [%]
1	70	9.86
2	140	8.40
3	830	5.80
4	1140	8.40

Inter-Assay (n=4)		
Probe	Carbonyl proteins [pmol/mg	Standard Deviation (SD) [%]
1	60	7.37
2	170	9.72
3	730	7.19
4	1130	6,36

Sensitivity

The detection limit was estimated to be 20 pmol/mg.

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.
- 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.

05.12.2013

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