

ACHTUNG: Geänderte Arbeitsanleitung (2015-06-15)

Bitte beachten:

- Standards und Kontrollen werden nicht mehr derivatisiert (Rekonstitutionsvolumen siehe Datenblatt)
- Geänderte Probenmenge vor Derivatisierung (Kapitel 6)









ATTENTION: Changed manual (2015-06-15)

Please pay attention to:

- No need to derivatize standards and controls (for volume of reconstitution see product specification)
- Changed sample volume for derivatization (chapter 6)

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

Verwendete Symbole:

	Temperaturbegrenzung		Bestellnummer
	In-Vitro-Diagnostikum		Zu verwenden mit
	Hersteller		Inhalt ausreichend für <n> Prüfungen
	Chargenbezeichnung		Verwendbar bis

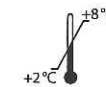
Manual

Carbonyl protein ELISA Kit

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

Valid from 2015-06-15

REF K 7870



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1. INTENDED USE

This ELISA Kit is intended for the determination of protein carbonyls in human serum and plasma. For *in vitro* diagnostic 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.

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	Holder with strips	12 x 8 wells
K 7870	WASHBUF	Wash buffer concentrate (10 fold)	1 x 100ml
K 7870	STD	Standards, lyophilized	4 x 5 vials
K 7870	CTRL 1	Control, lyophilized	4 x 1 vial
K 7870	CTRL 2	Control, lyophilized	4 x 1 vial

Cat. No.	Label	Kit components	Quantity
K 7870	CONJ	Conjugate concentrate, peroxidase-labelled	1 x 200 µl
K 7870	CONJBUF	Conjugate dilution buffer	1 x 15 ml
K 7870	AB	2nd antibody concentrate	1 x 200 µl
K 7870	ABBUF	Antibody dilution buffer	1 x 15 ml
K 7870	DER	Derivatization reagent	2 x 5 ml
K 7870	ASYBUF	Assay buffer	2 x 100 ml
K 7870	SUB	TMB substrate	1 x 15 ml
K 7870	STOP	Stop solution	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

- Ultra pure water*
- Calibrated precision pipettors and 0.5–1000 µl tips
- Foil to cover the microtiter plate
- Multi-channel pipets or repeater pipets
- Vortex
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* 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).

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:** Before use, the **wash buffer concentrate (WASHBUF)** must be diluted **1:10** with ultra pure water (100 ml WASHBUF + 900 ml ultra pure water), mix well. Crystals could occur due to high salt con-

centration in the stock solutions. The crystals must be redissolved at room temperature or 37 °C before dilution of the buffer solutions. 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 one month**.

- The **lyophilized standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. Before use, the **STD** (standards) and **CTRL** (controls) must be reconstituted as stated in the specification data sheet. **Reconstituted standards and controls are not stable and cannot be stored.**
- 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.
- **Preparation of the 2nd antibody:** The **AB** (2nd antibody concentrate) must be diluted **1:101 in ABBUF** (antibody dilution buffer): e.g. for one plate: 100 µl AB + 10 ml ABBUF. The **undiluted AB** is stable at **2–8 °C** until the expiry date stated on the label. **2nd antibody** (1:101 diluted AB) **is not stable and cannot be stored.**
- **Preparation of the conjugate:** The **conjugate concentrate (CONJ)** must be diluted **1:101** in conjugate dilution buffer (100 µl CONJ + 10 ml CONJBUF). The **CONJ** is stable at **2–8 °C** until 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 of test package) 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 to each tube 25 µl SAMPLE .
3.	Add to each tube 100 µl of DER (derivatization reagent).
4.	Close the tubes and vortex the content well.

5. For derivatization, incubate for **30 min at 37 °C***.

* Alternatively, incubate over night at 4 °C.

Sample dilution

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

- **30 µl** derivatized sample + **570 µl** assay buffer, mix well = **1:20 (dilution I)**
- **30 µl** dilution I + **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

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 second 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.

Test procedure

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

Mark the positions of STD/SAMPLE/CTRL (standards/samples/controls) on a protocol sheet.

Take as many microtiter strips as needed from kit. Store unused strips in the closed original 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.	Wash the coated microtiter plate 5 times with 250 µl wash buffer . After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution.
2.	For the analysis, pipet each 100 µl of STD, CTRL or SAMPLE (dilution III of samples) from into the respective well of the microtiter plate.
3.	Cover plate tightly and incubate for 1 hour at 37 °C .
4.	Aspirate the contents of each well. Wash 5 times by dispensing 250 µl 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 100 µl of 2nd antibody into each well.
6.	Cover the plate tightly and incubate for 1 hour at 37 °C .
7.	Aspirate the contents of each well. Wash 5 times by dispensing 250 µl 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 100 µl of conjugate into each well.
9.	Cover the plate tightly and incubate for 1 hour at 37 °C .
10.	Aspirate the contents of each well. Wash 5 times by dispensing 250 µl 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 100 µl of SUB (TMB substrate) into each well.
12.	Incubate for 10–20 min at room temperature in the dark*.
13.	Add 100 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 observing the color 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

The dilution factor of the plasma and serum samples is already taken into consideration in the serial dilution of the standards. Therefore no further multiplication of the obtained results is necessary.

9. LIMITATIONS

Samples with an OD higher than the OD of the highest standard should be further diluted and re-assayed. For the following analysis, the changed dilution factor has to be taken into consideration.

10. QUALITY CONTROL

Immundiagnostik 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 studies of matrix samples of apparently healthy persons (n = 41), a reference range 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
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 analyzed 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 color 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 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).
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 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).

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