

ACHTUNG: Geänderte Arbeitsanleitung

- Inhalt der Testpackung ohne STDBUF und SAMPLEBUF
- Proben mit ASYBUF verdünnen
- Erstellung der Standardkurve mit ASYBUF (siehe Spezifikationsdatenblatt)

ATTENTION: Changed manual

- Material supplied without STDBUF and SAMPLEBUF
- Samples have to be diluted with ASYBUF
- Creation of calibration curve with ASYBUF (see specification sheet)

Clinical and Investigative Medicine. Médecine Clinique et Experimentale **34** (3): E163-71.

7. Neubauer, Oliver, Daniel König, Norbert Kern, Lukas Nics, and Karl-Heinz Wagner. 2008. "No Indications of Persistent Oxidative Stress in Response to an Ironman Triathlon." *Medicine and Science in Sports and Exercise* **40** (12): 2119–28.

Verwendete Symbole:



Temperaturbegrenzung



Bestellnummer



Nur für Forschungszwecke



Zu verwenden mit



Hersteller



Inhalt ausreichend für <n> Prüfungen



Chargenbezeichnung



Verwendbar bis



Achtung

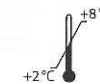
Manual

AOPP Kit

For the in vitro determination of Advanced oxidation protein products (AOPP) in EDTA plasma

Valid from 2016-12-15

REF K 7811W



RUO



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1. INTENDED USE

The described Assay is intended for the quantitative determination of advanced oxidation protein products (AOPPs) in EDTA-plasma. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Increased oxidative stress has been implicated in a wide range of diseases. In haemodialysis patients, massive generation of reactive oxygen species (ROS) is induced during each dialysis session. Proteins are highly susceptible to oxidative stress damage, which could result in formation of AOPPs (advanced oxidation protein products) or AGEs (advanced glycation end products, non-enzymatically glycosylated proteins with irreversible chemical modifications). AOPP measurement is proposed to be a reliable marker for the extent of protein oxidative damage in uremic patients. In addition, plasma AOPP determination may be useful for monitoring the effect of treatments with drugs reducing oxidative stress.

Possible research areas

- Monitoring of oxidative stress, e.g. in hemodialysed patients
- Monitoring of inflammatory processes
- Prognostic marker for progressive IgA nephropathy

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 7811W	PLATE	Microtiter plate	12x 8 wells
K 7811W	ASYBUF	Assay buffer, ready-to-use	1 x 65 ml
K 7811W	STDKONZ	Standard concentrate, lyophilised (see specification for concentration and preparation of a standard curve)	4x 1 vial
K 7811W	CTRL	Control, lyophilised (see specification for range)	2x 1 vial
K 7811W	DELIP	Delipidation reagent, ready-to-use	1x 2.5 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 10–1000 µl tips
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000g
- 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. STORAGE AND PREPARATION OF REAGENTS

- The **lyophilised concentrate** (STDKONZ) and **lyophilised control** (CTRL) are stable at **2–8 °C** until the expiry date stated on the label. **Reconstitution** details are given in the **specification data sheet**. **Standard concentrate and control** (reconstituted STDKONZ and CTRL) **are 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. PREPARATION OF SAMPLES

- Before analysis, **centrifuge** freshly collected **EDTA plasma** in 1.5 ml reaction tubes at 3 000 g for **30 s**
- Mix **125 µl centrifugated EDTA plasma** with **25 µl delipidation reagent** (DELIP), vortex → **dilution 1:1.2**
- Incubate for **10 min** at room temperature (15–30 °C)
- Afterwards, **centrifuge** at 3 000 g for **5 min**
- Mix **100 µl delipidated EDTA plasma** with **400 µl assay buffer** (ASYBUF) in an 1.5 ml reaction tube, vortex → **final dilution 1:6**

7. ASSAY PROCEDURE

Principle of the test

The assay is based on the spectroscopic analysis of modified proteins at 340 nm. Standards, controls and patient samples assayed for AOPP are placed in each well of a 96-well microtiter plate. The absorbance is read at 340 nm. The chloramine T (CT) absorbance at 340 nm is linear within the range of 0 to 100 µmol/l, AOPP concentrations are expressed as CT equivalents. A dose response curve of the absorbance unit at 340 nm vs. concentration is generated, using the values obtained from the standard. AOPP, present in the patient samples, is determined directly from this curve.

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 covered 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.

Attention

Standards, controls and samples must be pipetted without air bubbles.

Test procedure

1.	Add each 200 µl standards/controls/diluted samples into the respective wells.
2.	Determine directly the absorption of standards, control and samples at 340 nm .

8. RESULTS

The estimated AOPP value must be multiplied by the **dilution factor 6** to obtain the concentration in the patient samples.

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

9. 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

We recommend each laboratory to establish its own reference range.

10. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n = 16)

The precision (intra-assay variation) was calculated from 16 determinations on each one of three samples.

Sample	AOPP mean value [$\mu\text{mol/l}$]	CV [%]
1	28.7	5.6
2	171.9	1.3
3	522.3	3.0

Inter-Assay (n = 12)

Sample	AOPP mean value [$\mu\text{mol/l}$]	CV [%]
1	84.0	14.3
2	72.5	16.6

11. PRECAUTIONS

- All reagents in the kit package are for research use only.
- If human materials have been used in kit components, they 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.

12. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- 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.

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for 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 sent to Immundiagnostik AG along with a written complaint.

14. REFERENCES










General literature

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3. Descamps-Latscha, Béatrice, Véronique Witko-Sarsat, Thao Nguyen-Khoa, Anh Thu Nguyen, Valérie Gausson, Nadya Mothu, Camila Cardoso, et al. 2004. "Early Prediction of IgA Nephropathy Progression: Proteinuria and AOPP Are Strong Prognostic Markers." *Kidney International* **66** (4): 1606–12.
4. Nguyen-Khoa, T, Z A Massy, J P De Bandt, M Kebede, L Salama, G Lambrey, V Witko-Sarsat, T B Drüeke, B Lacour, and MThévenin. 2001. "Oxidative Stress and Haemodialysis: Role of Inflammation and Duration of Dialysis Treatment." *Nephrology, Dialysis, Transplantation : Official Publication of the European Dialysis and Transplant Association - European Renal Association* **16** (2): 335–40.

Literature using K 7811W

5. Gumieniczek, Anna, Beata Owczarek, and Bernadeta Pawlikowska. 2012. "Oxidative/nitrosative Stress and Protein Damages in Aqueous Humor of Hyperglycemic Rabbits: Effects of Two Oral Antidiabetics, Pioglitazone and Repaglinide." *Experimental Diabetes Research* **2012** (January): 653678.
6. Tabak, Omur, Remise Gelisgen, Hayriye Erman, Fusun Erdenen, Cüneyt Muderrisoğlu, Hale Aral, and Hafize Uzun. 2011. "Oxidative Lipid, Protein, and DNA Damage as Oxidative Stress Markers in Vascular Complications of Diabetes Mellitus." *Clinical and Investigative Medicine. Médecine Clinique et Experimentale* **34** (3): E163–71.
7. Neubauer, Oliver, Daniel König, Norbert Kern, Lukas Nics, and Karl-Heinz Wagner. 2008. "No Indications of Persistent Oxidative Stress in Response to an Ironman Triathlon." *Medicine and Science in Sports and Exercise* **40** (12): 2119–28.

Used symbols:

	Temperature limitation		Catalogue Number
	For research use only		To be used with
	Manufacturer		Contains sufficient for <n> tests
	Lot number		Use by
	Attention		