

Manual

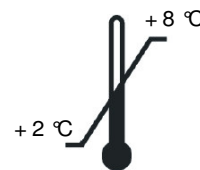
AOPP Kit

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

Valid from 15.05.2006



K 7811w



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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. It is for research use only.

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.

Indications

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

3. MATERIAL SUPPLIED

Catalogue No	Content	Kit Components	Quantity
K 7811w MTP	PLATE	Holder with strips	12 x 8 wells
K 7811w PV	SAMPLEB UF	Sample dilution buffer	50 ml
K 7811w SV	STDBUF	Standard dilution buffer	15 ml
K 7811w ST	STD	Standard concentrate, lyophilized (100 µmol/l CT eq; CT eq = Chloramine T equivalents)	2 vials
K 7811w KO	CTRL	Control, lyophilized	1 vial
K 7811w DR	DELIP	Delipidation reagent	1,5 ml

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Bidistilled water (aqua bidest.)
- Precision pipettors and disposable tips to deliver 10-1000 µl
- Multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 340

5. PREPARATION AND STORAGE OF REAGENTS

- **SAMPLEBUF** (sample dilution buffer) and **STDBUF** (standard dilution buffer) are ready-to-use.
- **DELIP** (delipidation reagent) is ready-to-use.
- **CTRL** (Control) must be reconstituted with 1 ml bidistilled water. Reconstituted control is stable for 2 months when stored at -20° C.
- **STD** (standard concentrate) 100 µmol/l CT eq must be reconstituted with 1 ml bidistilled water.
- **Standard curve** solution must be prepared from the **STD** (standard concentrate, 100 µmol/l CT eq) in **1:2** dilution steps by adding **STDBUF** (standard dilution buffer) as follows:

Standard concentrate 100 µmol/ml CT eq = S1

0,5 ml S1 + 0,5 ml STDBUF = S2 (50 µmol/l)

0,5 ml S2 + 0,5 ml STDBUF = S3 (25,0 µmol/l)

0,5 ml S3 + 0,5 ml STDBUF = S4 (12,5 µmol/l)

0,5 ml S4 + 0,5 ml STDBUF = S5 (6,25 µmol/l)

STDBUF (standard dilution buffer) is used as **standard 0 µmol/l**.

- 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. SAMPLE PREPARATION

Centrifugate fresh collected EDTA-Plasma in 1,5 ml cups at 10 000 rpm for 30 sec before analysis

Mix 125 µl centrifugated EDTA-Plasma with 25 µl DELIP (delipidation reagent), vortex

Dilution 1:1,2

Incubate for 10 min at room temperature

Afterwards, **centrifugate** at 10 000 rpm for 5 min

Mix 100 µl delipidated EDTA-Plasma with 400 µl SAMPLEBUF (sample dilution buffer) in 1,5 ml cup, 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 were placed in each well of a 96-well microtiter plate. The absorbance was read at 340 nm. The chloramine-T (CT) absorbance at 340 nm being linear within the range of 0 to 100 µmol/l, AOPP concentrations were expressed as CT equivalents.

Test procedure

1. Bring all reagents and samples to room temperature (18-26 °C) and mix well
2. Mark the positions of STD/SAMPLE/CTRL (Standards/Sample/Control) in duplicate on a protocol sheet
3. Add 200 µl STD (100 µmol/l; 50 µmol/l; 25 µmol/l; 12,5 µmol/l; 6,25 µmol/l) in duplicate into respective well
4. Add 200 µl STDBUF (Standard dilution buffer) as standard 0 µmol/l in duplicate into respective well
5. Add 200 µl Sample in duplicate into respective well
6. Add 200 µl CTRL in duplicate into respective well
7. Determine directly the absorption of standards and samples at 340 nm
8. Determine the AOPP concentration in diluted samples directly from the linear standard curve

8. RESULTS

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

9. QUALITY CONTROL

Immundiagnostik AG recommends the use of commercial control samples for internal quality control if available.

Control samples should be analyzed 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.

Expected values

Normal ranges

We recommend each laboratory to establish its own norm concentration range.

10. PRECAUTIONS

- For research use only.
- Quality control guidelines should be followed.
- 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.

11. TECHNICAL HINTS

- Do not mix different lot numbers of any kit component.
- Reagents should not be used beyond the expiration date shown on the 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.
- The assay should always be performed according the enclosed manual.

12. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for research use only.
- 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 wrong use.
- Warranty claims and complaints in respect of 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.

13. REFERENCES

1. Deschamps-Latscha B et al. (2005) Advanced oxidation protein products as risk factors for atherosclerotic cardiovascular events in nondiabetic predialysis patients. *Am J Kidney Dis* **45**(1):39-47
2. Deschamps-Latscha B et al. (2004) Early prediction of IgA nephropathy progression: proteinuria and AOPP are strong prognostic markers. *Kidney International* **66**: 1606-1612
3. Nguyen-Khoa T et al. (2001) Oxidative stress and haemodialysis: role of inflammation and duration of dialysis treatment. *Nephrol Dial Transplant* **16**: 335-340
4. Witko-Sarsat V et al. (1996) Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney International* **49**: 1304-1313

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