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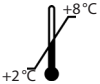
Manual

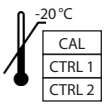
PerOx (TOS/TOC) Kit

Photometric test system for the determination of the total oxidative status/capacity (TOS/TOC) in EDTA plasma, serum and other biological samples

Valid from 2022-09-07

REF **KC5100**





IVD CE

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Table of Contents

1. INTENDED USE

This photometric Immundiagnostik assay is intended for the quantitative determination of the total oxidative status/capacity (TOS/TOC) in EDTA plasma, serum and cell culture supernatants. For *in vitro* diagnostic use only.

2. INTRODUCTION

An overproduction of oxygen radicals or insufficient antioxidative capacity leads to a dangerous imbalance in the organism. This starts pathological mechanisms which develop several diseases. The most important disease is the cardiovascular arteriosclerosis. Beside this inflammation processes, sepsis, cancerogenesis and neurodegenerative diseases are postulated.

This is the reason why the determination of the **oxidative status/oxidative stress** is of fundamental importance in medical diagnosis and in research. The methods used for measurement of radical-linked effects (lipid peroxidation) up to now are very time consuming (HPLC) or reflect only breakdown products of multiple unsaturated fatty acids (TBARS).

The **PerOx** assay is fast, reliable and easy to perform. **Total lipid peroxides** are measured. Because of a direct correlation between oxygen radicals and lipid peroxides, it is possible to measure and characterize the **oxidative status/oxidative stress** in biological fluids.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 0005.15	RECSOL	Reconstitution solution	15 ml
KC5100	CAL	Calibrator; lyophilised (see specification data sheet for concentration)	4 x
	CTRL1	Control 1; lyophilised	4 x
	CTRL2	Control 2; lyophilised	4 x
	REABUFA	Reaction buffer A	25 ml
	REABUFB	Reaction buffer B	1 ml
	ENZ	Enzyme solution	50 µl
	STOP	Stop solution	15 ml
	PLATE	Microtiter plate	1 x

Individual components can be ordered separately from Immundiagnostik. Please ask for the price list of the individual components.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Vortex
- Various pipettes
- Incubation chamber for 37 °C
- Microtiter plate reader (required filters see chapter 7)

5. STORAGE AND PREPARATION OF REAGENTS

Calibrator and controls

The **lyophilised calibrator** (CAL) and the **lyophilised controls 1 and 2** (CTRL1 and CTRL2) are stable at **-20 °C** until the expiry date stated on the label. Before use, they have to be reconstituted with each **250 µl reconstitution solution** (RECSOL). Allow the vial content to dissolve for 5 min and then mix thoroughly by vortexing. Aliquots of the **calibrator** (reconstituted CAL) and the **controls 1 and 2** (reconstituted CTRL1 and CTRL2) can be stored at **-20 °C** for **4 weeks**. **Avoid repeated thawing and freezing**. The concentration of calibrator and controls slightly changes from lot to lot. The exact concentration is stated on the label of CAL and in the specification of the controls, respectively.

Storage of the other reagents

Reaction buffer B (REABUF B) has to be stored at **2–8 °C in the dark**.

Test reagents are stable until the expiry date stated on the label when stored at **2–8 °C**.

Preparation of the reaction buffer mixture

To avoid losses, the enzyme solution (ENZ) should be centrifuged prior to use. After use, the vial has to be tightly closed to avoid contamination or evaporation (e.g. with parafilm).

In order to ensure the functionality of the **reaction buffer mixture**, preparation in light-protected containers (eg brown glass) is recommended.

The **reaction buffer mixture** must be prepared directly before use:

5 ml reaction buffer A (REABUF A)
+ **100 µl** reaction buffer B (REABUF B)
+ **5 µl** enzyme solution (ENZ)

Important: The amounts mentioned above are sufficient for 40 tests. For other sample numbers, the buffer volumes must be adjusted accordingly.

Important: The reaction buffer mixture **cannot be stored**. The reaction buffers (REABUF A, REABUF B) and the enzyme solution (ENZ) are stable at 2–8 °C until the expiry date stated on the label.

6. STORAGE AND PREPARATION OF SAMPLES

EDTA plasma and serum

Venous fasting blood is suited for this test system. EDTA plasma should be preferred because in serum, a time dependent increase in peroxide concentration is observed. EDTA plasma is stable at -20 °C for at least 4 weeks.

During serum preparation, it is important not to exceed 30 min at room temperature for clotting. Afterwards, the serum should be stored at -20 °C up to the measurement.

Samples with visible amounts of precipitates (mostly cryoproteins) should be centrifuged (at least 5 min at 10000 *g*) prior to measurement. The supernatant is used in the test.

Cell culture

In principle, it is possible to determine the PerOx concentration in cell culture supernatants. To test whether ingredients of the cell culture medium affect the measurement or not, we recommend the following approach:

- Dilute 10 µl of 30 % H_2O_2 concentrate with 1000 µl of reaction buffer A (REABUF A) = S_0.
- Dilute 5 µl of S_0 with 1000 µl of reaction buffer A (REABUF A) = S_1
- Preparation 1: Pipet 10 µl cell culture medium into one well.
- Preparation 2: Pipet 10 µl reconstitution solution (RECSOL) into another well.
- Add each 10 µl S_1 to both preparations, then add each 100 µl reaction buffer A (REABUF A) and 100 µl reaction buffer mixture (see preparation above). Incubate for 5 min. Add 50 µl stop solution (STOP) and measure immediately at 450 nm in a microtiter plate reader.

Evaluation of the cell culture medium results

A ratio of $OD_{preparation1}$: $OD_{preparation\ 2}$ > 0,8 demonstrates that there are no major disturbing factors in the tested cell medium, and the assay can be performed.

7. ASSAY PROCEDURE

Principle of the test

The determination of the peroxides is performed by the reaction of a peroxidase with peroxides in the sample followed by the conversion of TMB to a colored product.

After addition of a stop solution the samples are measured at 450 nm in a microtiter plate reader. The quantification is performed by the delivered calibrator.

Test procedure

The microtiter plate is ready to use.

Important: To ensure the reproducibility of the measurement, the given incubation time and temperature must strictly be followed.

1.	Pipet **10 µl** of sample, calibrator (CAL), controls (CTRL1, CTRL2) and blank/reconstitution solution (RECSOL) in appropriate wells.
2.	Add **100 µl** of reaction buffer A (REABUF A).
3.	**Measurement 1**: Read the absorption of the samples in the ELISA reader at 450 nm.
4.	Add **100 µl** of reaction buffer mixture.
5.	Incubate for **15 min** at **37 °C**.
6.	Add **50 µl** stop solution (STOP).
7.	**Measurement 2** is performed immediately after addition of the stop solution (STOP) at 450 nm in the ELISA reader.

8. RESULTS

The difference between measurement 1 and 2 is directly proportional to the peroxide content of the sample:

For evaluation, the estimated OD values of the first measurement are subtracted from the optical densities of the second measurement to obtain the ΔOD values of sample, calibrator, controls and blank.

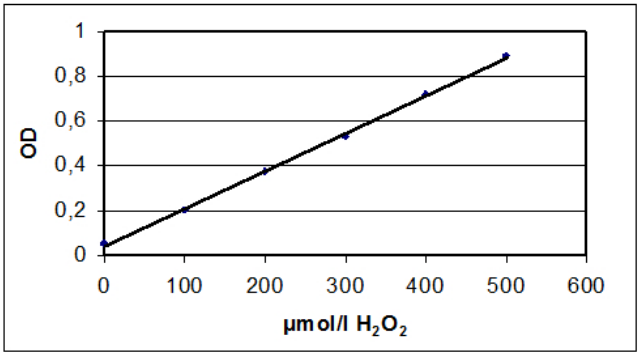
The concentrations of samples and controls are calculated using the calibrator (see label for concentration) and the following formula:

$$\text{Sample conc. [µmol/l]} = \frac{\Delta\text{OD sample} - \Delta\text{OD blank}}{\Delta\text{OD calibrator} - \Delta\text{OD blank}} \times \text{conc. calibrator [µmol/l]*}$$

* see label for concentration

A prepared Excel evaluation file can be requested by Immundiagnostik AG.

The following linear standard curve is for demonstrational purpose only. Because of the linearity in the chosen concentration range, one-point calibration using the included calibrator is sufficient.



9. LIMITATIONS

Whole blood cannot be used.

Strong haemolytic and lipaemic samples often show pathological concentrations. We do not recommend analysis of such samples.

The use of heparin plasma results in wrong results. Therefore, heparin plasma cannot be used in this assay.

10. QUALITY CONTROL

Reference ranges

EDTA-plasma

< 200 µmol/l	low oxidative stress
200–350 µmol/l	moderate oxidative stress
> 350 µmol/l	high oxidative stress

Serum

< 180 µmol/l	low oxidative stress
180–310 µmol/l	moderate oxidative stress
> 310 µmol/l	high oxidative stress

We recommend each laboratory to establish its own reference ranges. The values mentioned above are only for orientation and can deviate from other published data.

Controls

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.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-assay CV

2.94 % (162 µmol/l) [n = 6]

Inter-assay CV

6.63 % (136 µmol/l) [n = 10]

6.85 % (389 µmol/l) [n = 10]

Linearity

up to 800 µmol/l

Detection limit

7 µmol/l

12. PRECAUTIONS

- 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.
- The stop solution consists of diluted sulphuric acid (H_2SO_4), 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.
- The test components contain organic solvents. Contact with skin or mucous membranes has to be avoided.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are harmful to health and the environment. Substrates for enzymatic color reactions can also cause skin and/or respiratory irritation. Any contact with the substances should be avoided. Further safety information can be found in the safety
- As a precaution, it is recommended that the human material used is always considered potentially infectious.

13. GENERAL NOTES ON THE TEST

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- Do not interchange different lot numbers of any kit component within the same assay.
- Plugs and caps of different reagents should not be swapped.
- The guidelines for medical laboratories should be followed.
- Control samples should be analyzed with each run.
- The assay should always be performed according the enclosed manual.
- 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.
- Reagents should not be used beyond the expiration date stated on kit label.

- 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.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.

14. REFERENCES

1. Hildebrandt, W. et al., 2002. Effect of N-acetyl-cysteine on the hypoxic ventilatory response and erythropoietin production: linkage between plasma thiol redox state and O(2) chemosensitivity. *Blood*, **99**(5), pp.1552–5.
2. Reichenbach, J. et al., 2002. Elevated oxidative stress in patients with ataxia telangiectasia. *Antioxidants & redox signaling*, **4**(3), pp.465–9.
3. Schimke, I. et al., 2001. Decreased oxidative stress in patients with idiopathic dilated cardiomyopathy one year after immunoglobulin adsorption. *Journal of the American College of Cardiology*, (1), pp.178–83.

Used symbols: