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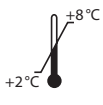
Manual

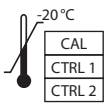
ImAnOx® (TAS/TAC) Kit

Photometric test system for the determination of the total antioxidative status/capacity (TAS/TAC) in serum and EDTA-plasma

Valid from 2022-09-07

REF **KC5200**





IVD CE

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1. INTENDED USE

This photometric assay is intended for the quantitative determination of the total antioxidative status/capacity (TAS/TAC) in EDTA-plasma and serum. For *in vitro* diagnostic only.

2. INTRODUCTION

The human body is constantly under attack from free radicals that occur as part of normal cell metabolism, and by exposure to environmental factors such as UV light, cigarette smoke, environmental pollutants and gamma radiation. The resulting „Reactive Oxygen Species" (ROS) circulates freely in the body with access to all organs and tissues, which can have serious repercussions throughout the body. The body possesses a number of mechanisms both to control the production of ROS and to cope with free radicals in order to limit or repair the damage to tissues

Overproductions of ROS or insufficient defense mechanisms lead to a dangerous disbalance in the organism. Thereby several pathomechanisms implicated in over 100 human diseases, e. g. cardiovascular disease, cancer, diabetes mellitus, inflammatory disease, aging, etc., were induced.

Determination of the antioxidative capacity becomes of fundamental importance in medical diagnosis and in research. The *ImAnOx*®-Assay is fast, reliable and easy to perform. The total antioxidative capacity is measured.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 0005.15	RECSOL	Reconstitution solution	15 ml
KC5200	PLATE	Microtiter plate	2 x
	CAL	Calibrator; lyophilised (see specification data sheet for concentration)	4 x
	CTRL1	Control1; lyophilised	4 x
	CTRL2	Control2; lyophilised	4 x
	PER	Peroxide solution	250 µl
	REABUFA	Reaction buffer A	105 ml
	REABUFB	Reaction buffer B	1.5 ml
	ENZ	Enzyme solution	50 µl
	STOP	Stop solution	15 ml

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 mixer
- Various pipettes
- Incubation chamber for 37 °C
- Microtiter plate reader

5. STORAGE AND PREPARATION OF REAGENTS

Calibrators 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

Test reagents are stable until the expiry date (see label of test package) when stored at **2–8 °C**.

Preparation of reagent 1

In order to ensure the functionality of reagent 1, it is important to use light-protected containers (eg brown glass) during preparation.

Reagent 1 has to be prepared directly before use. Peroxide solution (PER) is diluted in reaction buffer (READBUF).

For example:

5 ml reaction buffer A (READBUF A) + 10 µl peroxide solution (PER) = dilution 1

100 µl of dilution 1 + 4.9 ml reaction buffer A (READBUF A) = reagent 1

Please note: Reagent 1 is not stable and can not be stored.

Important: The amounts are sufficient for 50 tests (25 duplicates). For varying sample numbers, the buffer volumes must be adjusted accordingly.

Preparation of reagent 2

In order to ensure the functionality of reagent 2, it is important to use light-protected containers (eg brown glass) during preparation.

During the incubation, hydrogen peroxide generates reaction products which absorb at 450 nm. Due to this effect and self-absorption, it is important to measure **with and without addition of enzyme**.

Reagent 2 has to be prepared **directly before use**. Reaction buffer A (READBUF A) and reaction buffer B (READBUF B) are mixed with and without addition of enzyme solution (ENZ).

For example:

Reagent 2a: 5 ml reaction buffer A (READBUF A) + 100 µl reaction buffer B (READBUF B) + 5 µl enzyme solution (ENZ) = reagent 2 with enzyme solution

Reagent 2b: 5 ml reaction buffer A (READBUF A) + 100 µl reaction buffer B (READBUF B) = reagent 2 without enzyme solution

Please note: Reagent 2a with and reagent 2b without addition of enzyme solution (ENZ) is not stable and can not be stored.

I**mportant**: To avoid losses, the enzyme solution (ENZ) should be centrifuged prior to use. After use, the vial should be immediately and correctly closed to avoid contamination or evaporation (e. g. parafilm).

Important: The amounts are sufficient for 50 tests (25 duplicates). For varying sample numbers, the buffer volumes must be adjusted accordingly.

6. STORAGE AND PREPARATION OF SAMPLES

Serum and EDTA-plasma derived from venous fasting blood is suitable for this test system.

Lipaemia and haemolysis interfere with the test system. Such samples should not be measured.

Sample storage

The blood sample can be shipped at 2–8 °C within 24 hours. Serum and EDTA-plasma should be stored at -20 °C up to the measurement. They are stable at -20 °C for 4 weeks.

Preparation

Samples with visible amounts of precipitates should be centrifuged (5 min at 10000 *g*) prior to measurement, and the resulting supernatant is used in the test.

For testing in duplicates, pipette **4 x 10 µl** of each prepared sample per well.

7. ASSAY PROCEDURE

Principle of the test

The determination of the antioxidative capacity is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provided hydrogen peroxide (H_2O_2) The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H_2O_2 is determined photometrically by an enzymatic reaction which involves 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.

The difference between applied and measured peroxide concentration in a defined time period is proportional to the reactivity of the antioxidants of the sample (antioxidative capacity). Quantification is performed with the enclosed calibrator.

Please note: As the reaction speed of the distinct antioxidants in the sample is different, the measured concentrations of antioxidants are equivalent to the reactivity of the distinct antioxidants and not to their total amount in the sample. Therefore, we use in our test system hydrogen-peroxide equivalents as unit for the antioxidative capacity.

The concentration of total antioxidative status/capacity (TAS/TAC) can be quantified by referring the optical density of the calibrator.

We recommend to carry out the tests in duplicate.

Test procedure

Bring all **reagents and samples to room temperature** and mix well.

Mark the positions of CAL/SAMPLE/CTRL (calibrator/sample/controls) on a protocol sheet.

Example

	with enzyme		without enzyme		with enzyme		without enzyme		with enzyme		without enzyme	
	1	2	3	4	5	6	7	8	9	10	11	12
A	P1	P1	P1	P1	P9	P9	P9	P9	P14	P14	P14	P14
B	P2	P2	P2	P2	Ctrl1	Ctrl1	Ctrl1	Ctrl1	P15	P15	P15	P15
C	P3	P3	P3	P3	P10	P10	P10	P10	P16	P16	P16	P16
D	P4	P4	P4	P4	Cal	Cal	Cal	Cal	P17	P17	P17	P17
E	P5	P5	P5	P5	P11	P11	P11	P11	P18	P18	P18	P18
F	P6	P6	P6	P6	Ctrl2	Ctrl2	Ctrl2	Ctrl2	P19	P19	P19	P19
G	P7	P7	P7	P7	P12	P12	P12	P12	P20	P20	P20	P20
H	P8	P8	P8	P8	P13	P13	P13	P13	P21	P21	P21	P21

Px = patient sample, Cal = calibrator, Ctrl = control

Take as many microtiter strips as needed from kit. Store unused strips covered at 2–8 °C. Strips are stable until expiry date stated on the label.

1.	Add **10 µl of calibrator** (reconstituted CAL)**, sample or control 1 or 2** (reconstituted CTRL1 or CTRL2) into the respective wells. **Please note**: Always pipet samples, controls and calibrator in 4 wells, 2 wells for enzyme treatment and 2 without enzyme treatment.
2.	Add **100 µl** freshly prepared **reagent 1** in each well.
3.	Incubate for **10 min** at **37 °C**
4.	Add **100 µl** freshly prepared **reagent 2a (**with enzyme), respectively **reagent 2b** (without enzyme) in the appropriate wells.
5.	Incubate for **5 min** at **room temperature.**
6.	Add **50 µl** stop solution (STOP) and mix well.
7.	Determine **absorption immediately** with an microtiter plate reader at **450 nm**.

8. RESULTS

Calculation

The difference of the sample values with and without enzyme is inversely proportional to the antioxidative capacity:

To get the ΔOD, subtract the OD-values of samples without enzyme from the OD values of samples with enzyme. The antioxidative capacity is calculated according to the following formula:

$$\textit{antioxidative capacity of a sample [µmol/l]} = \textit{392 - (392 - calibrator concentration)} \times \frac{\Delta\,OD\ sample}{\Delta\,OD\ calibrator}$$

9. LIMITATIONS

The use of heparin plasma results in clouding of the starting solution and thus to incorrect measurement results. Strong haemolytic and lipaemic samples often show pathological antioxidant capacity. We therefore do not recommend the measurement of such samples. Whole blood is not suitable for the measurement.

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 EDTA-plasma and serum of apparently healthy persons (n = 69), the following reference values have been estimated:

low antioxidative capacity	< 280 µmol/l
middle antioxidative capacity	280-320 µmol/l
high antioxidative capacity	> 320 µmol/l
Mean value	305 µmol/l

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

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n = 12)

Sample	*ImAnOx*® [µmol/l]	CV [%]
1	213	3,99
2	308	2,04

Inter-Assay (n = 12)

Sample	*ImAnOx*® [µmol/l]	CV [%]
1	217	2,65
2	285	3,89

Analytical Sensitivity

Limit of detection 130 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.
- The guidelines for medical laboratories should be followed.
- *ImAnOx®* is a trademark of Immundiagnostik AG.
- 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.
- Do not interchange different lot numbers of any kit component within the same assay.
- Control samples should be analyzed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according the enclosed manual.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.

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
	Contains plasma derivatives or human blood		Consult instructions for use
	Consult specification data sheet		Do not re-use
	Unique Device Identification		Contains material of animal origin
	Medicinal substance		Contains material of human origin