OxyStat

Colorimetric assay for the quantitative determination of peroxides
in EDTA-plasma, serum and other biological fluids

Cat. No. BI-5007
96 tests

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1. Introduction

Cells and tissues are sensitive to oxidative stress, caused by the formation of free radicals. If not deactivated by antioxidants, organic peroxides and hydroperoxides are the first reaction products between cellular constituents and free radicals or other reactive oxygen derivates [1].
These peroxides cause numerous short- and longterm disorders, particularly cardiovasculary diseases and atherosclerosis, but they are also considered to be risk factors in inflammatory processes, sepsis, carcinogenesis and neurodegenerative processes [2,3,4].
The determination of the oxidative status / oxidative stress is essential in today's medical research and diagnostics. Methods used so far were either expensive (HPLC), or detected only degradation products of polyunsaturated fatty acids, like TBARS (thiobarbituric acid reactive substances).
The Biomedica OxyStat assay measures the total peroxide concentration of a sample, utilizing a quick and simple assay procedure. Results show a direct correlation between free radicals and circulating biological peroxides and thus allow the characterization of the oxidative status in biological samples.
For more information please visit the OxyStat section of our homepage http://www.biomedica.co.at/oxystat

2. Principle of the assay

The peroxide concentration is determined by reaction of the biological peroxides with peroxidase and a subsequent color-reaction using TMB as substrate. After addition of a stop solution, the colored liquid is measured photometrically at 450 nm.
A calibrator is used to calculate the concentration of circulating biological peroxides in the sample (one-point calibration).

3. Contents of the kit

- Microtiter plate
- Solution A, sample buffer, ready to use.
- Solution B, reaction buffer, lyophilized.
- Solution C, solvent buffer, ready to use.
- Solution D, enzyme solution, ready to use.
- Solution E, reconstitution solution, ready to use.
- Calibrator, 3 vials, lyophilized. The concentration after reconstitution is stated on the label.
- Stop solution, ready to use.
- Instructions for use (package insert).

All reagents are to be stored at 4 °C, until expiry date stated on the label.

4. Additional material and equipment required

Pipettes of 5 µl, 10 µl, 50 µl, 100 µl, 250 µl, 600 µl, 5ml
Vortex mixer,
Incubator for 37 °C
ELISA reader equipped with 450 nm filter

5. Reagents and sample preparation-performance of the assay

Sample stabilities:	2 - 8 °C	-20 °C
EDTA-plasma	48 h	5 weeks
serum	24 h	48 h

Heparin-plasma, lipemic or haemolytic samples may give erroneous results. Cloudy samples should be centrifuged at least 5 minutes at 5000 x g before used in the assay.
All samples should be mixed well before assaying.

Reagent preparation:

- Mark positions for calibrator, controls and samples on the protocol sheet supplied.
- Take microtiter strips out of the bag and mark as appropriate.
- All reagents should be kept at 4 °C until used in the assay.

Assay procedure manual

1. Dissolve lyophilized solution B in 600 µl solution C, vortex.
   This buffer is light-sensitive and should be stored in the dark. The solution is stable at 2-8 °C for 3 months.
2. Dissolve one calibrator in 250 µl solution E and leave at room temperature (18-26 °C) for five minutes, vortex.
3. The following volume of ABD-reaction-mix is sufficient for 40 tests (wells) and should be used as a guidance. However, the volume should be adjusted to the respective number of samples.
   Prepare the ABD-reaction-mix immediately before the assay by mixing:
   5 ml solution A + 100 µl solution B + 5 µl solution D.
4. Pipette 10 µl calibrator and samples into the respective wells.
5. Add 100 µl solution A into all wells.
6. Measurement 1: Determine absorption with an ELISA reader at 450 nm.
7. Add 100 µl of the ABD-reaction-mix into all wells.
8. Incubate 15 minutes at 37 °C.
9. Add 50 µl stop solution into all wells.
10. Measurement 2: Determine absorption with an ELISA reader at 450 nm

Assay procedure automat

1. Adjust volumes of the reaction solutions and samples according to the analyser used.
2. If the analyzer is incapable of adding the stop-solution (see page 6, point 9.), perform kinetic measurements at 405 nm or at 580-620 nm without stopping the reaction.
3. Measurement time: 15 minutes at 20-37 °C.

6. Assay procedure

Dissolve solution B in 600 µl solution C.
Dissolve one calibrator in 250 µl solution E.
Prepare ABD-reaction-mix (sufficient for 40 tests):
5 ml solution A + 100 µl solution B + 5 µl solution D.
⇩
Pipette 10 µl calibrator and samples into respective wells.
⇩
Add 100 µl solution A into all wells.
⇩
1. Measurement at 450 nm.
⇩
Add 100 µl ABD-reaction-mix.
⇩

Incubate 15 minutes at 37 °C.
⇩
Add 50 µl stop solution into all wells.
⇩
2. Measurement at 450 nm.

7. Calculation of results

The differences between measurements 1 and 2 are proportional to the peroxide concentrations of the samples.

1. For each calibrator, control and sample, subtract the OD-values of measurement 1 from the OD-values of measurement 2 (= ΔOD).

2. A single-point calibration is done using the calibrator. The ΔOD-value of the calibrator is proportional to its concentration, which is stated on the label. This concentration is stated as H_2O_2-equivalents (µmol/l).

3. The concentrations of controls and samples are calculated according to the following formula:

$$[\mu mol / l]\ sample = \frac{\Delta OD\ sample \times [\mu mol / l]\ calibrator}{\Delta OD\ calibrator}$$

8. Assay characteristics

Reference values from apparently healthy persons with no documented disease and medication:

EDTA-plasma: <400 µmol/l
Serum: <350 µmol/l
Each laboratory should establish its own range of reference values.

Precision:

Intraassay

mean	CV	n
221 µmol/l	3.1 %	12

Interassay

mean	CV	n
221 µmol/l	5.1 %	12

Linearity:
Linear up to 600 µmol/l

Detection limit:
7 µmol/l

Sample types:
EDTA-plasma, serum and other biological fluids

Sample volume:
10 µl/test

Storage:
4 °C

9. Technical hints

- To avoid cross-contamination, change pipette tips between addition of calibrator, controls, samples, buffers and stop solution. Also use separate reservoirs for each reagent!
- Do not mix stoppers and caps of different reagents - contamination!
- Do not use reagents beyond expiry date.
- Protect reagents from direct sunlight.
- Do not mix or substitute reagents with those from other lots or sources.

- Stop solution should be added to the plate in the same order as the reaction-mix solution.

10. Precautions

- Do not pipette by mouth.
- Do not eat, drink, smoke or apply cosmetics where reagents are used.
- Avoid all contact with the reagents by using gloves.
- Stop solution contains diluted sulphuric acid. Irritation to eyes and skin is possible.
  Flush with water after contact!

11. Literature

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April 2000