EAGLE BIOSCIENCES

Total Lipid Hydroperoxide ELISA

Catalog Number: PER31-K01
96 Wells
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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1. Intended use

The Eagle Biosciences Total Lipid Hydroperoxide ELISA kit is intended for the quantitative determination of peroxides in EDTA-plasma, serum and cell culture supernatants. The Total Lipid Hydroperoxide ELISA kit is for research use only and not to be used in diagnostic procedures.

2. Introduction

In a healthy body oxidative and reductive processes are in a balance. Free radicals (reactive oxygen species) are eliminated by antioxidants. In case of a lack of antioxidants, free radicals react with cell structures. A reaction with unsaturated fatty acid leads to lipid peroxidation products. These circumstances are under discussion during the genesis of atherosclerosis. Recent findings suggest that free radicals are also involved in inflammation, sepsis, carcinogenesis and neurodegenerative diseases.

An increased level of peroxides in blood indicates oxidative stress in the body. The measurement of peroxides can indicate an increased risk for heart attack and stroke. A subsequent change in nutrition and/or supplementation therapy might reduce the risk.

The Total Lipid Hydroperoxide ELISA kit allows an easy, rapid and precise quantitative determination of the total peroxides in biological samples. The kit includes all reagents ready to use for preparation of the samples.

3. Warnings and precautions

- All reagents of this kit are strictly intended for Research Use Only.
- Do not interchange kit components from different lots.
- The stop solution (STOP) contains acid and has to be handled carefully. It is corrosive and causes burns. It should be handled with gloves, eye protection, and appropriate protective clothing in a hood. Any spill should be wiped out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation. In case of an accident or indisposition contact immediately a physician.
- The substrate TMB (tetramethyl benzidine) is toxic by ingestion and contact with the skin. Any spill should be wiped out immediately with copious quantities of water.
- Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
- The reagents of the Total Lipid Hydroperoxide ELISA kit contain bactericides to protect against bacterial growth. Avoid the contact with the skin or mucous membrane.

- Reagents should not be used beyond the expiration date shown on kit label.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

4. Materials Provided

Catalog #	Component	Description	Amount
IC5100ka	CAL	Calibrator (lyoph. 0.25 ml)	3 vials
IC5100ko	CTRL	Control 1 and 2 (lyoph. 0.25 ml)	2 vials each
IC5100ra	REABA	Reaction buffer A	25 ml
IC5100rb	REABB	Reaction buffer B	1 ml
IC5100el	ENZYM	Enzyme solution	50 µl
IC5100re	RECON	Reconstitution solution	5 ml
IC5100sl	STOP	Stop solution **(caution: corrosive)**	6 ml
IC5100mp	MTP	Microtiter plate	1 piece

5. Additional special equipment

- Laboratory balance
- Centrifuge, 3000xg
- Glass or plastic vials
- Various pipettes
- Foil to cover the microtiter plate
- Multichannel or multi-pipette
- ELISA reader with filter 450 nm
- Microtiter plate shaker
- Vortex mixer

6. Reagent preparation

- Reconstitute the **calibrator (CAL)** and the **controls (CTRL)** in **250 µl reconstitution solution (RECON)**. After 5 minutes they should be homogenized on a vortex mixer. If

necessary, divide them in several portions and store them at -20°C (the reconstituted calibrator and controls are stable for at least 2 weeks at –20°C). Avoid repeated freeze-thaw circles. The concentration might have minor changes from lot to lot. All other test reagents are stable at 2-8 °C, up to the date of expiry stated on the label.

- **Preparation of the reaction buffer mixture:** The reaction buffer mixture is prepared just before usage.

  5 ml REABA + **100 µl** REABB + **5 µl** ENZYM.

  The REABB should be stored in the dark.

  Important: The given amounts are sufficient for 40 tests (20 duplicates). Please correct for your sample amount.

  Important: The enzyme solution should be centrifuged prior to usage to remove liquid which might be at the lid. After usage the vial should be sealed with sealing film (e.g. Parafilm).

  *NOTE: The Reaction Buffer Mixture **cannot** be stored.*

7. Specimen

Venous fasting blood can be used in this test system. EDTA-plasma is preferred because in serum a time dependent increase in peroxide concentration is possible. During preparation of serum it is important not to exceed 30 min at room temperature for clotting. Serum should be stored at –20 °C up to the measurement. EDTA-plasma is stable at – 20 °C for 2 weeks. Samples with visible amounts of precipitates should be centrifuged (5 min at 10000 g) prior to measurement. The resulting supernatant can be used in the test.

8. Procedure

Principle of the method

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.

Sample preparation

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

1. Pipette **10 µl** Sample, CAL and CTRL in duplicates in appropriate wells.
2. Pipette **100 µl** REABA in appropriate wells.
3. **Measurement 1**: Read the absorption of the samples in the ELISA reader at 450 nm.
4. Add **100 µl** reaction buffer mixture to appropriate wells.
5. Incubate for **15 min** at **37° C**.
6. Add **50 µl** STOP to appropriate wells.
7. **Measurement 2**: Read the absorption immediately after addition of the stop solution (STOP) at 450 nm in the ELISA reader.

9. Calculation of analytical results

Calculation

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

- For evaluation, the optical densities of Measurement 1 are subtracted from the optical densities of Measurement 2.
- Samples and controls are then calibrated by the use of the calibrator (concentration is given on the label). The concentration of the calibrator is given in H_2O_2-equivalents (µmol/l).

$$\text{Conc. of the sample} = \frac{\Delta\text{OD sample} * \text{Conc. calibrator}}{\Delta\text{OD calibrator}}$$

10. Internal quality control

Reference values

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 that each laboratory should develop their own normal range. The values mentioned above are only for orientation and can deviate from other published data.

11. Validation data

Precision and reproducibility

Intra-Assay CV:	2.0 % (168 µmol/l)	[n = 6]
	3.6 % (596 µmol/l)	[n = 6]
Inter-Assay CV:	3.6 % (168 µmol/l)	[n = 6]
	3.5 % (608 µmol/l)	[n = 6]

Linearity

up to 1000 µmol/l

Detection limit

6 µmol/l

12. Limitations of the method

Heparin plasma leads to turbidity and therefore to false high results. Whole blood or strong hemolytic and lipemic samples often show pathological concentrations. These samples must not be measured.

13. Literature references

Schimke I, et al. J Am Coll Cardiol 2001;38:178-83.
Hildebrandt W, et al. Blood 2002;99:1552-5
Reichenbach et al. Antioxid redox signal 2002; 4: 465-69

For further information about this kit, its application or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.