invitron
high sensitivity diagnostics

IV3-003E

Ox-LDL/MDA Adduct ELISA Kit

For in-vitro diagnostic use only

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Definitions

 Instructions for use

REF Catalogue number

 Use by

LOT Lot/Batch Code

 Storage temperature limitations

IVD In vitro diagnostic medical device

 Contains sufficient for <N> tests

Invitron Ox-LDL/MDA Adduct ELISA Kit

Intended Use

The Invitron ox-LDL ELISA Kit is intended for the quantitative assay of ox-LDL in EDTA-plasma and serum. The test recognises MDA-modified apolipoprotein B 100 containing less than 60 MDA units per molecule. The kit is for *in vitro* diagnostic use only.

Summary and Explanation

Lipid peroxidation is a natural process that is essential for normal cell growth. However, when oxidative stress is sufficient to overwhelm the antioxidative cell defence, the normal balance is disturbed and enhanced formation of lipid peroxidation products occurs. Currently, lipid peroxidation is considered to be a basic mechanism involved in the initiation and progression of a range of diseases. For example, there is considerable evidence suggesting that oxidative stress resulting in increased lipid peroxidation and protein modification is involved in the pathogenesis of atherosclerosis and coronary heart disease.

Lipid peroxidation products are formed as a consequence free radicals reacting with unsaturated fatty acids, in particular those in low-density lipoprotein (LDL), the major carrier of blood cholesterol. LDL is eliminated through the action od macrophages. Normally, receptor-mediated uptake of LDL is reduced by down-regulation of LDL receptor expression in response to increasing cholesterol levels. Once LDL is oxidised, it is still internalised by macrophages but through the action of scavenger receptors whose expression is not controlled by cholesterol loading. The binding of ox-LDL induces cholesterol accumulation by macrophages, transforming them into lipid-loaded “foam cells”. This process is accompanied by cell proliferation and the release of extracellular matrix components and contr5ibutes to the genesis and progression of atherosclerosis by inducing endothelial damage and by amplifying the inflammatory response within the vessel wall.

Foam cells have been found in the earliest detectable atherosclerotic lesions – the precursors to the complex atherosclerosis that results in arterial stenosis and reduced blood flow. Such advanced lesions ultimately provide sites for thrombosis resulting in myocardial infarction.

Principle

The Invitron ox-LDL assay is an ELISA for the direct measurement of ox-LDL in human EDTA-plasma and serum. Standards, controls and samples are introduced into the wells of a microtitre plate coated with specific antibodies. Captured analyte is quantified by means of a second antibody labelled with peroxodase. The activity of the bound enzyme is determined by the use of tetramethylbenzidine (TMB). The intensity of the yellow colour produced following addition of a stop reagent is a function of the amount of bound labelled antibody and hence the concentration of ox-LDL in the sample. The concentration is determined by comparison with the colour produced by known standards.

Materials Provided

- Coated Microtitre Plate (a)
  (8 x 12) stripwells coated with specific antibody.
- Labelled Antibody Concentrate (b)
  (150 µl) goat-anti ox-LDL peroxidise labelled.
- Labelled antibody diluent (c)
  (15 ml) buffer for diluting peroxidise-labelled antibody
- Standards (d-h)
  (4 x 5 vials) lyophilised standards.
- Controls (i,j)
  (4 x 2 vials) lyophilised low and high controls.
- Substrate (k)
  (15 ml) Tetramethylbenzidine (TMB) substrate ready to use.
- Stop Solution (l)
  (15 ml) Dilute sulphuric acid, ready to use.
- Sample Diluent (m)
  (50 ml) ready to use.
- Wash Buffer Concentrate (n)
  (2 x 100 ml) 10x concentration phosphate buffered saline containing detergent and preservative.

Materials Required but not Supplied

- Deionised water
- Calibrated pipettes capable of delivering 10 to 1000 µl
- Plate sealers
- Microtitre plate shaker
- Multi-channel or repeating dispenser
- Vortex mixer
- Microtitre plate reader

Warnings and Precautions

- For *in vitro* diagnostic use only.
- Human materials used in the kit components have tested negative for HIV, hepatitis B and hepatitis C. Nevertheless, for safety reasons, all components should be treated as potentially infective.
- Stop solution is diluted sulphuric acid and therefore must be handled with care. It can cause burns and so gloves, safety glasses and appropriate protective clothing should be worn when handling it. Any spills should be wiped up immediately using large volumes of water.
- Reagents should not be used beyond the expiry date shown on the kit label.

Preparation and Storage of Reagents

- To run the assay more than once, refer to the kit storage conditions indicated on the label. Prepare only the required quantity of reagents for each run. Up to 4 runs may be performed with each kit.
- Reagent vials containing a volume of less than 100 µl should be centrifuged before use.
- Wash buffer concentrate should be diluted 1:10 in deionised water before use. If any crystals have formed during storage, these should be re-dissolved by warming the wash buffer to 37ºC prior to dilution. Wash buffer concentrate is stable when stored at 2-8º up to the expiry date on the label. Diluted wash buffer can be stored in a closed vessel for up to 4 weeks at 2-8ºC.
- Lyophilised standards and controls must be reconstituted with 0.5 ml deionised water before use. Allow the vials to stand for 10 minutes and then mix thoroughly by gentle inversion. **Reconstituted standards and controls are not stable and cannot be stored.**
- The labelled antibody concentrate must be diluted 1:100 with labelled antibody diluent. Undiluted labelled antibody concentrate is stable at 2-8ºC until the expiry date. **Diluted labelled antibody is not stable and cannot be stored**.
- All other test reagents are ready to use. These reagents are stable until the expiry date given on the kit label when stored at 2-8ºC.

Specimen Collection and Preparation

EDTA-Plasma and Serum

- The test is best performed on fasting samples. Samples may be stored for up to 2 years frozen at -20ºC.
- Lipaemic or haemolysed samples may give erroneous results and should not be used.
- Samples with visible signs of precipitation should be centrifuged before use.
- Samples should be diluted 1/10 with Sample Dilution Buffer prior to analysis.

Assay Procedure

1. Bring all components to room temperature before use.
2. Wash the required number of wells 5 times with working strength Wash Buffer (250 µl/well).
3. Add 100 µl of standard, control or sample to the appropriate well. It is recommended that all determinations are carried out in duplicate.
4. Cover with a plate sealer and incubate for 2 hours at room temperature on a plate shaker.
5. Discard the contents of the wells by inversion and wash the plate 5 times with working strength Wash Buffer (250 µl/well).
6. Add 100 µl working strength labelled antibody to each well.
7. Incubate for 1 hour at room temperature on a plate shaker.
8. Discard the contents of the wells by inversion and wash the plate 5 times with working strength Wash Buffer (250 µl/well).
9. Add 100 µl TMP substrate to each well.
10. Incubate for 10-20 minutes at room temperature in the dark.
11. Add 100 µl Stop Solution to each well.
12. Measure absorption at 450 nm in an ELISA plate reader.

Results

The results may be calculated automatically using a cubic spline or 4-parameter curve fit. The concentration of the samples can be read directly from this standard curve. For calculation of the concentration of ox-LDL in EDTA-plasma or serum, results should be multiplied by 10 to account for the dilution factor.

Limitations

- Only if test instructions are rigidly followed will optimum results be achieved.
- Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as thorough mixing of all prepared solutions.
- While rinsing, check that all wells are filled evenly with wash buffer, and that there are no residues in the wells.

Quality Control

The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. It is also recommended to make use of national or international Quality Assessment programs where possible in order to ensure the accuracy of the results. Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. If, after checking the above mentioned items, there is no error, contact Invitron directly.

Expected Values

In a study of a healthy population in Germany, a mean concentration of ox-LDL of 287 ng/ml (range 18-2261) was found.

It is recommended that each laboratory establishes its own reference range.

Performance

Linearity

Two patient samples were diluted with sample buffer and analysed. The results are shown in the following table.

Sample	Dilution	Expected ox-LDL (ng/ml)	Measured ox-LDL (ng/ml)
A	1/15	3503.00	3503.00
	1/30	1751.50	1827.50
	1/60	875.75	920.25
	1/120	477.50	437.88
B	1/40	7867.00	7867.00
	1/80	3933.50	3868.00
	1/160	1966.75	2000.75
	1/320	983.38	952.63

Sensitivity

Sensitivity of the Invitron ox-LDL assay, calculated as 2SD above the zero standard, was 4.13 ng/ml.

For additional information and product support please contact:

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