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	See external label	2°C-8°C	Σ=96 tests	REF #2032-14

OLAB

Enzyme immunoassay for the quantitative
determination of human autoantibodies against oxidized
low-density lipoprotein in serum

Cat. No.2032-14

1. Introduction

Oxidized low density lipoprotein (oLDL) is believed to play a critical role in the development and progression of atherosclerosis (1-3). Accumulation of oLDL in macrophages and smooth muscle cells causes foam cell formation, an initial step in the disease (4-6). Recent evidence suggests that autoantibodies against oxidatively modified LDL can be used as a parameter that consistently mirrors the occurance of oxidation processes taking place in vivo. In fact, elevated levels of autoantibodies against oLDL have been detected in the blood stream of patients with coronary artery disease (7). Moreover, recent studies indicate a correlation between autoantibodies against oLDL and the progression of carotid atherosclerosis (8). Increased serum concentrations of oLAB have also been described in various diseases such as pre-eclampsia and systemic lupus erythematosus (9, 10). Decreased oLAB titers were observed during septicemia and myocardial infarction (11, 12).
An overview on the clinical applications of oLAB has recently been published (11).

2. Principle of the assay

The oLAB kit is an enzyme immunoassay designed to determine human autoantibodies to oxidized LDL directly in serum. Cu ++ oxidized LDL is coated onto microtiter strips as antigen (13, 14). Autoantibodies, if present in the prediluted serum, bind specifically to the antigen. After a washing step, a specific peroxidase conjugated anti human IgG antibody detects the presence of bound autoantibodies. After removal of unbound conjugate through washing, tetramethylbenzidine (TMB) is added to the wells as a non toxic chromogenic substrate. The concentration of specific IgG in the sample is quantitated by an enzyme catalized colochange detectable on an standard ELISA reader. The amount of color develeoped is drectly proportional to the concentration of antibodies in the sample. The assay is standardized with defined amounts of oLAB in a serum matrix. The IgG concentration in the samples is quantitated in milliunits (mU), based on a characterized serum with a high oLAB titer and a low cross reactivity with native LDL (< 5%).

3. Contents of the kit

Store all reagents refrigerated at 4 °C. Refer to the expiry date on the kit box.

1 uncoated microwell plate for sample dilution

2 x 6 microwell strips
each 8 well strip is coated with oxidized LDL; packed in alubag with desiccant; 1 stripholder

Assay buffer
The vial contains 120 ml assay buffer ready for use

Washing buffer
The vial contains 100 ml of 10 fold buffer concentrate

Conjugate
The vial contains 12 ml mouse monoclonal anti-human IgG fc specific horseradish peroxidase conjugate, ready for use

6 standards
The vials contain 1200, 600, 300, 150, 75, 37 mU/ml oLAB in 0,5 ml human serum, ready for use

2 controls
The vials contain about 1000 and 300 mU/ml oLAB in 0,5 ml human serum, ready for use

Substrate
The vial contains 12 ml TMB solution, ready for use

Stop solution
The vial contains 7 ml of stop solution, ready for use (diluted sulphuric acid)

2 self-adhesive plastic films

1 protocol sheet

1 package insert (instructions for use)

4. Additional material and equipment required

Distilled water
Measuring cylinder
Pipettes for 200 µl and 50 µl with disposable tips
Multichannel pipette or multipette for 20 µl, 100 µl and 200 µl
Incubator for 37 °C
Manual or automatic microwell washer
ELISA reader equipped with 450 nm filter
Semilogarithmic paper or software for calculation of results

5. Sample preparation

Store freshly collected serum samples at -20 °C if not assayed on the same day. Do not use lipemic or hemolytic samples. This test system is not designed for plasma.
Preparation of reagents:

Allow all reagents to reach room temperature (18 - 26 °C) before use

Dilute washing buffer concentrate 1:10 (1 part buffer + 9 parts distilled water) to a final volume of 1000 ml with distilled water. Diluted buffer is stable at 4 °C until expiry date stated on the label.

Store coated strips in a desiccator after removal from the foil pouch. Strips will be stable at room temperature (18-26 °C) until expiry date stated on the label.

6. Performance of the assay

We recommend duplicates for all values. Standards, controls and samples must be prediluted 1:50 according to the following protocol:

Mark positions for blank, standards, controls and samples on the protocol sheet supplied

Take microtiter strips out of the alubag (plate 1) and mark as appropriate. Mark two wells as blank

Mark uncoated microwell plate exactly in the same way (plate 2 - for predilution)

Pipette 200 µl of assay buffer into all marked wells of the coated microwell plate (plate 1) incl. blank

Pipette 200 µl of assay buffer into all marked wells of the uncoated microwell plate (plate 2)

Pipette 50 µl of standard, control or sample in respective wells of plate 2

Shake well

Transfer 20 µl of the prediluted samples to the coated strips (Plate 1) with a multichannel pipette
THIS STEP MUST BE COMPLETED WITHIN 15 MINUTES!

Cover strips with plastic film and incubate for 90 minutes at 37 °C

Discard contents of the wells and wash 4 x with 300 µl diluted washing buffer

Add 100 µl conjugate to all wells including blank

Cover strips with plastic film and incubate for
30 minutes at room temperature

Discard contents of the wells and wash 4 x with 300 µl diluted buffer

Add 100 µl substrate to all wells including blank

Incubate strips for 15 minutes at room temperature in the dark

Add 50 µl stop solution to all wells

Shake well and determine absorption with an ELISA reader at 450 nm against 690 or against 620 nm as reference

7. Calculation of results

The extinction of the blank is substracted from all other values. A calibration curve is constructed from the standards. Commercially available software can be used as well as semilogarithmic paper. Results of the samples are read from this calibration curve. If the concentration of oLAB in the sample exeeds 1100 mU/ml, further dilution and measurement of the diluted sample is recommended.

8. Assay characteristics

Standard range: 37 - 1200 mU/ml

Sample: 50 µl serum

Assay time: 3 hours

Storage: 4 °C

Precision:Intraassay

Mean value (mU/ml)	**CV**	**Number**
119 ± 4	3,6%	n = 8
324 ± 14	4,3%	n = 8

Interassay

Mean value (mU/ml)	CV	Number
139 ± 11	8,2%	n = 5
544 ± 22	4,0%	n = 5

9. Precautions

All test components of human source were tested with 3rd generation tests against HIV-Ab and HBsAG; all components were found to be negative. However, standards as well as controls should be handled and disposed as if they were infectious, since no test method can offer complete assurance.

Do not interchange kit components from different lots

Do not use kit components beyond the expiry date

Protect reagents from direct sunlight

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

Sulphuric acid is irritating to eyes and skin. Flush with water if contact occurs

All liquid reagents contain 0,01% thimerosal as preservative. Avoid contact with skin and mucous membrane.

10. References

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14. Tatzber F. et al. (1991), Atherosclerosis 89: 203 – 208

11. Incubation scheme

	Blank	Standard/Controls	Sample
Buffer	200 µl	200 µl	200 µl
Standards/Controls		20 µl	
Samples			20 µl

incubate the microwell strips covered with plastic film for 90 min. at 37 °C in an incubator
discard liquid and wash the wells 4 times with 300 µl washing solution

Conjugate	100 µl	100 µl	100 µl

incubate the microwell strips covered with plastic film for 30 min. at room temperature
discard liquid and wash the wells 4 times with 300 µl washing solution

Substrate	100 µl	100 µl	100 µl

incubate for 15 min. at room temperature in the dark

Stop solution	50 µl	50 µl	50 µl

read results with an ELISA microwell reader at 450 nm and
620 or 690 nm as a reference

* - prediluted (1 + 4)

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