OLAB IgG

ANTI OXIDIZED LOW DENSITY LIPOPROTEIN

ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF
HUMAN IgG AUTOANTIBODIES AGAINST OXIDISED LOW DENSITY LIPOPROTEIN IN SERUM
CAT. NO. BI-20032. 12 X 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

CONTENT

Additional information on our products is available on our website.

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EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com

1) INTRODUCTION

Oxidized low density lipoprotein (oLDL) is believed to play a critical role in the development and progression of atherosclerosis. Accumulation of oLDL in macrophages and smooth muscle cells causes foam cell formation, an initial step in the disease. Autoantibodies against oxidatively modified LDL can be used as a parameter that consistently mirrors the occurrence 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. Moreover, recent studies indicate a correlation between autoantibodies against oLDL and the progression of carotid atherosclerosis. Increased serum concentrations of oLAB have also been described in various diseases such as pre-eclampsia and systemic lupus erythematosus. Decreased oLAB titers were observed during septicemia and myocardial infarction.
An overview on the clinical applications of oLAB has been published.

POSSIBLE INDICATIONS

- atherosclerosis
- coronary artery disease
- risk stratification from myocardial infarction
- acute septicemia

2) CONTENTS OF THE KIT

CONT	KIT COMPONENTS	QUANTITY
PLATE	Oxidised LDL coated microtiterstrips in stripholder packed in alu bag with desiccant	12 x 8 tests
ASYBUF	Assay buffer, red cap, ready to use	1 x 60 ml
WASHBUF	Wash buffer concentrate 20x, natural cap	1 x 50 ml
STD	Standards (37, 75, 150, 300, 600, 1,200 mU/ml oLAB IgG), white caps, ready to use	6 x 500 µl
CTRL	Controls (300, 1,000 mU/ml), yellow caps, ready to use	2 x 500 µl
CONJ	Conjugate, (monoclonal anti human IgG-HRPO), amber cap, ready to use	1 x 13 ml
SUB	Substrate (TMB solution), blue cap, ready to use	1 x 13 ml
STOP	Stop solution H_2SO_4, white cap, ready to use	1 x 7 ml

3) ADDITIONAL MATERIAL ADDED TO THE KIT

- 2 self-adhesive plastic films
- 1 uncoated microtiter plate for sample dilution
- QC protocol
- Protocol sheet
- Instruction manual for use

4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Incubator for 37°C
- Precision pipettes calibrated to deliver 50 µl and 200 µl and disposable tips
- Multichannel pipette or Multipipette for 20 µl, 100 µl and 200 µl
- ELISA reader for absorbance at 450 nm (or from 450 nm to 620 nm)
- Graph paper or software for calculation of results
- Plate washer is recommended for washing
- Distilled or deionised water

5) REAGENTS AND SAMPLE PREPARATION

Samples should be stored at -20°C if not assayed on the same day, long term storage at -70°C. Do not use lipemic or haemolysed samples. Samples should be mixed well before assaying. The OLAB IgG (Anti Oxidized Low Density Lipoprotein) ELISA Assay Kit is not designed for plasma.

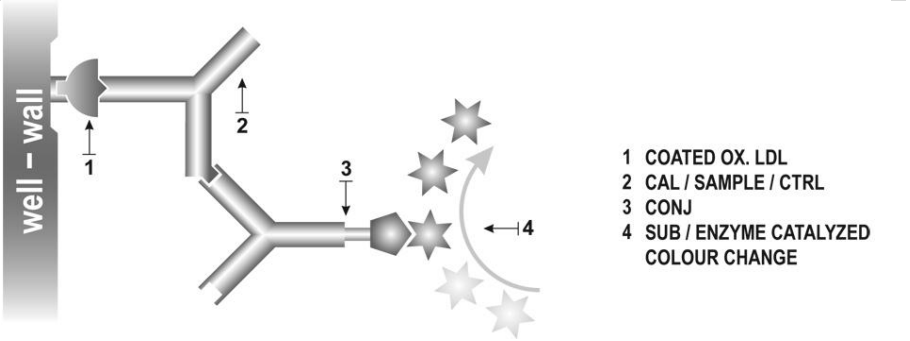
WASHBUF (Wash buffer): Dilute the concentrate 1:20 (e.g. 50 ml WASHBUF + 950 ml distilled water). Crystals in the buffer concentrate will dissolve at room temperature. Diluted buffer is stable at 2-8°C until expiry date stated on label.
Use only diluted WASHBUF (Wash buffer) for the assay performance.

PREDILUTION:
All STD/SAMPLE/CTRL (standard/sample/control) must be used in 1:55 end-dilution (pre-dilution 1:5 + assay-dilution 1:11) in the assay. Use the enclosed uncoated microtiter plate for the 1:5 pre-dilution step.

- Add 200 µl ASYBUF (Assay buffer) into the appropriate wells of the uncoated microtiter plate.
- Add 50 µl STD/SAMPLE/CTRL (standard/sample/control) into the wells, mix well (=1:5 dilution)

Note: Pre-diluted material must be used in the assay within 15 minutes.
Follow the ASSAY PROTOCOL

6) PRINCIPLE OF THE ASSAY



7) ASSAY PROTOCOL

All reagents and samples must be at room temperature (18-26°C) before use in the OLAB IgG (Anti Oxidized Low Density Lipoprotein) ELISA Assay Kit.
Mark position for BLANK/STD/SAMPLE/CTRL (Blank/Standard/Sample/Control) on the protocol sheet.
Take microtiter strips out of the alu bag, take a minimum of one well as Blank. Store unused strips with desiccant at 2-8°C in the alu bag. Strips are stable until expiry date stated on the label.

Add 200 µl ASYBUF (Assay buffer) into respective wells of the coated microtiter strips, including blank.
Add 20 µl 1:5 pre-diluted STD/SAMPLE/CTRL into each well except blank, swirl gently. ATTENTION: The transfer of the pre-diluted STD/SAMPLE/CTRL into the coated microtiter strips must be completed within 15 minutes. Use a Multichannel pipette.
Cover tightly and incubate for 1.5 hours at 37°C.
Aspirate and wash wells 4x with 300 µl diluted WASHBUF (Wash buffer), remove remaining WASHBUF by hitting plate against paper towel after the latest wash.
Add 100 µl CONJ (Conjugate) into each well except blank.
Cover tightly and incubate for 30 minutes at room temperature (18-26°C).
Aspirate and wash wells 4x with 300 µl diluted WASHBUF (Wash buffer), remove remaining WASHBUF by hitting plate against paper towel after the latest wash.
Add 100 µl SUB (Substrate) into each well.
Incubate for 15 min at room temperature (18-26°C) in the dark.
Add 50 µl STOP (Stop solution) into each well.
Measure absorbance immediately at 450 nm with reference 620 nm, if available.

8) CALCULATION OF RESULTS

Subtract the blank extinction from all other values. Construct the standard curve from the standard values. Use commercially available software or graph paper. Obtain sample concentration from this standard curve. Respective dilution factors have to be considered.
If the concentration of oLAB IgG in the sample exceeds 1,100 mU/ml, further dilution and measurement of the diluted sample is recommended.

The quality control protocol supplied with the kit shows the results of the final release QC for each kit. Data for optical density obtained by customers may differ due to various influences and/or due to the normal decrease of signal intensity during shelf life. However, this does not affect validity of results as long as an optical density of 1.00 or higher is obtained for the standard with the highest concentration.

9) ASSAY CHARACTERISTICS

Normal range:	Median: 263 mU/ml (n = 50) Each laboratory has to establish its own reference range for the samples under investigation.
Standard range:	37 to 1,200 mU/ml
Sample volume:	50 µl human serum
Detection Limit:	48 mU/ml (37 mU/ml + 3x SD)
Incubation time:	1.5 h / 30 min / 15 min

10) PRECISION

Intra-Assay (n=8)		
Mean (mU/ml)	119	324
SD	4	14
CV%	3.6%	4.3%

Inter-Assay (n=5)		
Mean (mU/ml)	139	544
SD	11	22
CV%	8.2%	4%

11) TECHNICAL HINTS

- Do not mix or substitute reagents with those from other lots or sources.
- Do not mix stoppers and caps from different reagents or use reagents between lots.
- Do not use reagents beyond expiration date.
- Protect reagents from direct sunlight.
- Substrate solution should remain colourless until added to the plate.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.

12) PRECAUTIONS

All test components of human source were tested with 3rd generation tests against HIV-Ab and HBsAg and were found negative. Nevertheless, they should be handled and disposed as if they were infectious.Liquid reagents contain ≤0.1% Proclin 300 as preservative.

Avoid contact with skin and mucous membrane. Proclin 300 is not toxic in concentrations used in this kit. It may cause allergic skin reactions, avoid contact with skin or eyes.

- Do not pipette by mouth.
- Do not eat, drink, smoke or apply cosmetics where reagents are used.
- Avoid all contact with reagents by using gloves.
- Sulfuric acid is irritating to eyes and skin. Avoid contact with skin and mucous. Irritations are possible – Flush with water if contact occurs!

13) LITERATURE

- "Evaluation of the atherogenic tendency of lipids and lipoprotein content and their relationships with oxidant-antioxidant system in patients with psoriasis"
  Vanizor Kural B. et al. Clin Chim Acta 2003 Feb;328(1-2):71-82
- "Circulating autoantibodies to oxidized LDL correlate with impaired coronary endothelial function after cardiac transplantation"
  Fang JC et al. Arterioscler Thromb Vasc Biol 2002 Dec 1;22(12):2044-8
- "Autoantibodies to malondialdehyde-modified low-density lipoprotein in patients with angiographically confirmed coronary artery disease"
  McDowell A. et al. J Pharm Pharmacol 2002 Dec;54(12):1651-7

SYMBOLS

Expiry date / Verfallsdatum / Date de péremption / Data di scadenza /Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Udløbsdato / Utgångsdatum / Termin Ważności / Lejárati idő / Doba exspirácie / Doba exspirace

Consider instructions for use / Bitte Gebrauchsanweisung beachten / Consultez la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las instrucciones de utilización / Consulte as instruções de utilização / Raadpleeg de gebruiksaanwijzing / Se brugsanvisningen / Läs anvisningarna före användning / Proszę przeczytać instrukcję wykonania / Vegyük figyelembe a használati utasításban foglaltakat / Postupujte podľa pokynov na použitie / Postupujte dle návodu k použití

In vitro Diagnostic Medical Device (for in Vitro Diagnostic Use)/ In vitro Diagnostikum (zur In-vitro-Diagnostik) / Dispositif médical de diagnostic in vitro (Pour usage diagnostique in vitro) / Dispositivo medico per diagnostica in vitro (per uso diagnostico in vitro) / Dispositivo médico de diagnóstico in vitro (para uso diagnóstico in vitro) / Dispositivo médico para diagnóstico in vitro (Para utilização de diagnóstico "in vitro") / Medisch hulpmiddel voor diagnostiek in vitro (Voor diagnostisch gebruik in vitro) / Medicinsk udstyr til in vitro-diagnostik (Udelukkende til in vitro diagnostisk anvendelse) / Medicinteknisk produkt avsedd för in vitro-diagnostik (För in vitro-diagnostiskt bruk) / Wyrób medyczny do Diagnostyki In Vitro / In vitro orvosdiagnosztikai termék / In vitro diagnostický zdravotnícky materiál (určené pre diagnostiku „in vitro“) / In vitro diagnostický zdravotnicky materiál (určeno pro diagnostiku „in vitro“)

Lot-Batch Number / Charge-Chargennummer / Lot-Code du lot / Lotto-Numero di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Batchkode / Lot-Satskod / Numer serii / Lot-Batch szám / Číslo šarže / Číslo šarže

Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por / Vervaardigd door / Fabrikation af / Tillverkad av / Wyprodukowane pr / Gyártotta / Vyrobené / Vyrobeno

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Store at between / Lagerung bei zwischen / Conserver à entre / Conservare a tra / Conservar a temp. entre / Armazene a entre / Bewaar bij tussen / Opbevares mellem / Förvaras vid / Przechowywać w / Tároljuk …… között / Skladujte v rozsahu / Skladujte v rozmezí

Contains sufficient for x tests / Inhalt ausreichend für x Tests / Contient suffisant pour x tests / Contenuto sufficiente per x test / Contiene suficiente para x pruebas / Contém suficiente para x testes / Bevat voldoende voor x bepalingen / Indeholder tilstrækkeligt til x prøver / Innehållet räcker till x analyser / Zawartość na x testów / Tartalma X teszt elvégzésére elegendő / Obsahuje materiál pre x testov / Obsahuje materiál pro x testů

BI-20032 OLAB IgG

ASSAY PROTOCOL AND CHECKLIST

- ❑ Bring all reagents to room temperature (18-26°C).
- ❑ Prepare reagents and samples as instructed.
- ❑ Bring unused and prepared components to the storage temperature mentioned in the package insert.
- ❑ Take coated microtiter strips out of the alu bag and mark positions on the protocol sheet.
- ❑ Add 200 µl ASYBUF (Assay buffer) into each well of the coated microtiterstrips.
- ❑ Add 20 µl 1:5 prediluted STD/SAMPLE/CTRL into each well except blank, swirl gently. Use a multichannel pipette. This step must be completed within 15 minutes from the predilution.
- ❑ **Cover tightly and incubate for 90 minutes at 37°C.**
- ❑ Aspirate and wash wells with 300 µl WASHBUF (Wash buffer) four times. Remove remaining buffer by hitting plate against paper towel.
- ❑ Add 100 µl CONJ (Conjugate) into each well except blank.
- ❑ **Cover tightly and incubate for 30 minutes at room temperature (18-26°C).**
- ❑ Aspirate and wash wells with 300 µl WASHBUF (Wash buffer) four times. Remove remaining buffer by hitting plate against paper towel.
- ❑ Add 100 µl SUB (Substrate) into each well.
- ❑ **Incubate for 15 minutes at room temperature (18-26°C), in the dark.**
- ❑ Add 50 µl STOP (Stop solution) into each well.
- ❑ Read optical density at 450 nm with reference 620 nm, if available.

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

Manufactured by:

BIOMEDICA

Biomedica Medizinprodukte GmbH & Co KG
A-1210 Wien, Divischgasse 4
Tel.+43/1/291 0745 • Fax +43/1/291 0785
www.bmgrp.com • export@bmgrp.com

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EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
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