MDA – Oxidized LDL

ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF MDA-OXIDISED LDL
IN HUMAN SERUM, CITRATE PLASMA, EDTA PLASMA OR HEPARIN PLASMA
CAT. NO. BI-20022. 12 X 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
CONTENT / INHALT

1. ENGLISH ........ 3

Additional information on our products is available on our website.

www.bmgrp.com

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1) INTRODUCTION

Oxidatively modified lipoproteins (ox-LDLs) play an important role in the progression of atherosclerosis and coronary artery disease. Low-density lipoprotein (LDL), the main carrier of plasma cholesterol, consists of a hydrophobic core and a surface monolayer of polar lipids and Apolipoprotein-B (ApoB). Oxidative stress and the consequent formation of free radicals lead to the peroxidation of ApoB. Malondialdehyde (MDA) has been identified as one of the major lipid peroxidation products of LDL, thus playing an important role in the LDL oxidation.

The Biomedica MDA ox-LDL ELISA Assay Kit specifically detects MDA-modified Apo B in human serum and plasma.

2) CONTENTS OF THE KIT

<table>
<thead>
<tr>
<th>CONT</th>
<th>KIT COMPONENTS</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLATE</td>
<td>Anti oxLDL antibody, microtiter plate strips in stripholder packed in alubag with desiccant</td>
<td>12 x 8 tests</td>
</tr>
<tr>
<td>WASHBUF</td>
<td>Wash buffer concentrate 20x, natural cap</td>
<td>1 x 50 ml</td>
</tr>
<tr>
<td>ASYBUF</td>
<td>Assay buffer, red cap, ready to use</td>
<td>1 x 13 ml</td>
</tr>
<tr>
<td>STD</td>
<td>Standards (0; 0.62; 1.25; 2.5; 5; 10 µg/ml), white caps, lyophilised</td>
<td>6 x 150 µl</td>
</tr>
<tr>
<td>CTRL</td>
<td>Control, white cap, lyophilised, exact concentration see label</td>
<td>1 x 150 µl</td>
</tr>
<tr>
<td>CONJ</td>
<td>Conjugate, (anti oxLDL antibody-HRPO), amber cap, ready to use</td>
<td>1 x 13 ml</td>
</tr>
<tr>
<td>SUB</td>
<td>Substrate (TMB solution), blue cap, ready to use</td>
<td>1 x 13 ml</td>
</tr>
<tr>
<td>STOP</td>
<td>Stop Solution, white cap, ready to use</td>
<td>1 x 7 ml</td>
</tr>
</tbody>
</table>

3) ADDITIONAL MATERIAL ADDED TO THE KIT

- 2 self-adhesive plastic films
- QC protocol
- Protocol sheet
- Instruction manual for use

4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 20 µl, 50 µl, 100 µl, 300 µl and disposable tips
- ELISA reader for absorbance at 450 nm (or from 450 nm to 630 nm)
- Plate washer is recommended for washing, alternatively multichannel pipette or manifold dispenser
- Refrigerator with 4°C (2-8°C)
- Graph paper or software for calculation of results
- Distilled or deionised water

5) REAGENTS AND SAMPLE PREPARATION

All reagents of the MDA Oxidized LDL ELISA Assay kit are stable at 4°C (2-8°C) until the expiry date stated on the label of each reagent.

Sample preparation:
We recommend separating plasma or serum by centrifugation as soon as possible, e.g. 20 min at 2000 x g, preferably at 4°C (2-8°C). Aliquot the acquired plasma or serum samples and store them at -25°C or lower. Samples should be mixed well before assaying. We recommend duplicates for all values. Lipemic or haemolysed samples may give erroneous results. Samples with values above highest STD could be diluted with STD0 or oxLDL negative human serum. Dilutions up to 1:10 are recommended.

For further information on sample stability please visit our website www.bmgrp.com technical file or contact our customer service by e-mail export@bmgrp.com or by phone +43/ 1/ 29107-45.
Reconstitution/Handling:
STD (standards) and CTRL (control): Pipette 150 µl of deionised or distilled water into each vial. Leave at room temperature (18-26°C) for 20 min. Swirl gently. The concentration is printed on the label. Reconstituted STD and CTRL are stable at -25°C or lower until expiry date. Avoid more than 4 freeze-thaw cycles.

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 4°C (2-8°C) until expiry date stated on label. Use only diluted WASHBUF (Wash buffer) for the assay performance.

6) PRINCIPLE OF THE ASSAY

7) ASSAY PROTOCOL
All reagents and samples must be at room temperature (18-26°C) before use in the assay.
Mark position for BLANK/STD (Standards)/SAMPLE/CTRL (Control) on the supplied protocol sheet.
Take microtiter strips out of the aluminium bag, take a minimum of one well as blank. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium bag. Strips are stable until expiry date stated on the label.

1. Add 100 µl ASYBUF (Assay buffer) into each well.
2. Add 20 µl STD/CTRL/SAMPLE (Standard/Control/Sample) in duplicate into respective wells, except blank.
3. Cover the strips tightly and incubate for 90 min at room temperature (18-26°C).
4. Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer). Remove remaining WASHBUF by hitting plate against paper towel after the last wash.
5. Add 100 µl CONJ (Conjugate) into each well, except blank.
6. Cover the strips tightly and incubate for 90 min at room temperature (18-26°C).
7. Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer). Remove remaining WASHBUF by hitting plate against paper towel after the last wash.
8. Add 100 µl SUB (Substrate) into each well.
9. Incubate for 30 min at room temperature (18-26°C) in the dark.
10. Add 50 µl STOP (Stop solution) into each well, shake well.
11. Measure absorbance immediately at 450 nm with reference 630 nm, if available.
8) CALCULATION OF RESULTS

Read the optical density (OD) of all wells on a plate reader using 450 nm wavelength (correction wavelength 630 nm). Subtract the blank OD from the values of STD, CTRL and sample. Construct the standard curve from the OD values of the STD. Use software or graph paper. Obtain sample concentration from this standard curve. The assay was evaluated with a 4PL algorithm. Different curve fitting methods need to be evaluated by the user. Respective dilution factors have to be considered.

Example typical STD-curve:

The quality control (QC) protocol supplied with the kit shows the results of the final release QC for each lot at production date. Data for OD 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 OD of 1.0 or more is obtained for the STD with the highest concentration and the value of the CTRL is in range (target range see label).

9) ASSAY CHARACTERISTICS

Values from apparently healthy individuals: Serum samples of 71 blood donors had a median level 1.0 µg/ml. Each laboratory should establish its own reference range for the samples under investigation.
Standard range: 0 – 10 µg/ml
Sample volume: 20 µl human serum or plasma (Citrate, EDTA or Heparin)
Detection Limit: (0 µg/ml + 3SD): 0.05 µg/ml
Incubation time: 90 min / 90 min / 30 min

For further information on assay characteristics please visit our website www.bmgrp.com technical file or contact our customer service by e-mail export@bmgrp.com or by phone +43/ 1/ 29107-45.

10) PRECISION

Intra-Assay: 3 samples of known concentrations were tested 3 times
Preliminary Inter-Assay: 3 samples of known concentrations were tested in 1 assay from 2 different operators

<table>
<thead>
<tr>
<th></th>
<th>Intra-Assay (n=3)</th>
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<tbody>
<tr>
<td></td>
<td>Mean (µg/ml)</td>
<td>0.625</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>SD (µg/ml)</td>
<td>0.05</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>CV%</td>
<td>7.2</td>
<td>8.4</td>
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<table>
<thead>
<tr>
<th></th>
<th>Inter-Assay</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean (µg/ml)</td>
<td>0.625</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>SD (µg/ml)</td>
<td>0.09</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>CV%</td>
<td>13.5</td>
<td>6.7</td>
</tr>
</tbody>
</table>

CV%
11) TECHNICAL HINTS

- Do not mix or substitute reagents with those from other lots or sources of the MDA Oxidized LDL ELISA Assay kit.
- Do not mix stoppers and caps from different reagents or use reagents between lots.
- Do not use reagents beyond expiration date of the MDA Oxidized LDL ELISA Assay kit.
- 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 of the MDA Oxidized LDL ELISA Assay kit.

12) PRECAUTIONS

All test components of the MDA Oxidized LDL ELISA Assay kit 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. 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

**SYMBOLS**

- Expiry date / Verfallsdatum / Date de péremption / Data di scadenza / Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Udgångsdatum / Termin Ważności / Lejárati idő / Doba expiracíe / Doba exspirace

- Consider instructions for use / Bitte Gebrauchsanweisung beachten / Consultez la notice d'utilisation / Consultare le istruzioni per l'uso / 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 peragnostica in vitro (per uso diagnostic in vitro) / Dispositivo médico para diagnostico in vitro (Para utilização de diagnostic "in vitro") / Medisch hulpmiddel voor diagnostiek in vitro (Voor diagnostisch gebruik in vitro) / Medicinsk udstyr til in vitro-diagnostik (Udelukkende til i vitro diagnostisk anvendelse) / Medicinteknik produkt avsedd för in vitro-diagnostik (För in vitro-diagnostisk bruk) / Wyrób medyczny do Diagnostyki In Vitro / In vitro orvosdiagnosztikai termék / In vitro diagnostický zdravotnický materiál (určené pre diagnostiku „in vitro“) / In vitro diagnostický zdravotnický 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 / Vervaardig door / Fabrikation af / Tillverkad av / Wyprodukowane pr / Gyártotta / Vyrobené / Vyrobeno

- Catalogue Number / Bestellnummer / Numéro de référence / Numero di riferimento / Número de referencia / Número de referência / Referentienummer / Referencenummer / Numer katalogowy / Katalogüsszám / Katalogové číslo / Katalogové číslo

- 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 Teste / Contient suffisant pour x tests / Contenido suficiente para x pruebas / Contêm suficiente para x testes / Bevat voldoende voor x bepalingen / Indeholder tilstrækkeligt til x prøver / Innehåller räcker till x analyser / Zawartość na x testów / Tartalma X teszt elvégzésére elegendő / Obsahuje materiál pre x testov / Obsahuje materiál pro x testů
BI-20022 MDA-oxLDL
ASSAY PROTOCOL AND CHECKLIST

PREPARATION OF REAGENTS:

- 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 microtiter strips out of the aluminium bag and mark positions on the protocol sheet.

TEST PROCEDURE:

- Add 100 µl ASYBUF (Assay buffer) into each well.
- Add 20 µl STD/CTRL/SAMPLE/ (Standard/Control/Sample) into respective wells, except blank.
- Cover tightly and incubate for 90 min at room temperature (18-26°C).
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- Add 100 µl SUB (Substrate) into each well.
- Incubate for 30 min at room temperature (18-26°C), in the dark.
- Add 50 µl STOP (Stop solution) into each well, shake well.
- Read Optical Density at 450 nm with reference 630 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.