Malondialdehyde (MDA) HPLC Assay

Catalog Number: MDA31-H100
100 Tests
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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1. Intended purpose

The Eagle Biosciences Malondialdehyde (MDA) HPLC Assay kit is intended for the quantitative determination of malondialdehyde in plasma and serum. The Malondialdehyde (MDA) HPLC Assay kit is for research use only and is not for use 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 of free radicals with unsaturated fatty acids leads to lipid peroxidation products. A reaction of free radicals with poly-unsaturated fatty acids generates malondialdehyde and/or 4-hydroxynonenal. These secondary lipid peroxidation products might react with other molecules in the cell. Modifications of the DNA-based adenine or guanine result in incorrect transcripts. A reaction of free radicals with proteins leads to an alteration or loss of function. The creation of neoantigens is possible. Neoantigens are recognized by the immune system, thus resulting in autoimmune diseases. The participation of lipid peroxidation products have been discussed in several diseases such as atherosclerosis, tumor genesis, rheumatism and reperfusion injury after transplantation.

The Eagle Biosciences Malondialdehyde (MDA) HPLC Assay kit makes it possible to determine the lipid peroxidation product in an easy, fast and precise method. The Malondialdehyde (MDA) HPLC Assay kit includes all reagents ready to use for preparation and separation of the samples with exception of the columns (IC1900rp) and the controls (IC1900ko). Both can be supplied by Eagle Biosciences. Beside the complete test kits it is possible to order all components separately. Please request our single component price list.

3. Warnings and precautions

- All reagents of this Malondialdehyde (MDA) HPLC Assay kit are strictly intended for research use only.
- The Malondialdehyde (MDA) HPLC Assay kit and column are concerted. Using alternative columns might cause in insufficient separation, resulting in false high results. The given test characteristics might not be fulfilled.
- Do not interchange the Eagle Biosciences Malondialdehyde (MDA) HPLC Assay kit components from different lots.
- Calibrator and controls contain human serum. It was tested and found negative for HBsAg, anti-HIV-1/2, and anti-HCV. No test can guarantee the absence of

HBsAg or HIV, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.

- The derivatisation solution (DERIVAT) 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 reaction solution (REAL) and mobile phase (ELU) contain organic solvents and have to be handled carefully. Organic solvents are highly flammable and toxic if inhaled or contact the skin. They 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.
- Wear disposable gloves while handling specimens or Malondialdehyde (MDA) HPLC Assay 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.
- 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 Malondialdehyde (MDA) HPLC Assay kit reagents, controls and serum samples observe the existing legal regulations.

4. Materials Provided

Article no.	Component	Designation	Amount
IC1900lm	ELU	Mobile phase	1000 ml
IC1900ka	CAL	Calibrator, (lyoph. 0.25 ml)	5 vials
IC1900dl	DERIVAT	Derivatisation solution	100 ml
IC1900rb	REAL	Reaction solution	50 ml

5. Additional special equipment

- 1.5 ml reaction tubes (Eppendorf)
- Centrifuge
- Various pipettes
- HPLC with Fluorescence-detector
- HPLC column malondialdehyde (IC1900rp)
- Heatable shaker or water bath
- Vortex mixer

6. Reagent preparation

- Reconstitute the **calibrator (CAL)** in **0.25 ml** deionized water. Divide it in several portions and store them at -20 °C. Avoid repeated freeze-thaw circles. The concentration of malondialdehyde might have minor changes from lot to lot.
- All other Malondialdehyde (MDA) HPLC Assay kit reagents are delivered ready to use.
- The test reagents are stable at 2-8 °C, the calibrator at -20 °C up to the date of expiry given on the label.

7. Specimen

- EDTA-plasma and serum from fasting blood can be used in this test system.
- The samples have to be protected from light, cooled and centrifuged immediately.
- The samples are stable in the dark at 2-8°C for 24 hours. For longer storage samples should be frozen at -20 °C.

8. Procedure

Principle of the method

For the determination of malondialdehyde a derivatisation step, in which protein bound malondialdehyde is hydrolyzed and converted into a fluorescent probe (60 min at 95 °C) is performed. The fluorescent probe is then cooled (2-8°C), centrifuged, mixed with a reaction solution and injected into the HPLC system. The isocratic separation via HPLC at 30°C, using a „reversed phase“ column, lasts 4 minutes for one sample. The chromatograms are recorded by a fluorescence detector. The quantification is performed with the delivered calibrator; the concentration is calculated via integration of the peak heights.

Sample preparation

Important: During the derivatisation reaction a fluorescent compound is produced which interferes with the malondialdehyde peak. Therefore it is necessary to run a deionized water blank which is subtracted from all samples.

1. Pipette into 1.5 ml reaction tubes:

   20 µl sample, CAL, CTRL or blank (deionized water)

   +

   1 ml DERIVAT

2. Mix well (**15 seconds** on a vortex mixer),
3. Incubate for **60 minutes at 95°C** on a shaker or in a water bath;
4. Keep the incubation time exactly because only for these conditions the given MDA-concentrations for calibrator and controls are valid.
5. Cool the samples to 2-8°C and centrifuge at 10.000g for 5 minutes
6. Mix 500 µl of the supernatant + 500 µl REAL
7. Inject 20 µl of the supernatant for chromatography into the HPLC-system. The supernatant is stable in the dark for 4 days at 2-8°C and 12 hours at 20-25 °C.

Chromatographic conditions

Column material:	Bischoff Prontosil Eurobond, 5 µm	
Column dimension:	125 mm x 4 mm	
Flow rate:	0.8-1.0 ml/min	
Fluorescence detection:	Excitation	515 nm
	Emission	553 nm
Injection volume:	20 µl	
Running time:	4 min	
Temperature:	30 °C	

Important: The mobile phase must not be re-circulated.

Treatment of the HPLC column

After the analysis the column should be flushed with 15 ml deionized water(1 ml/min) and stored in 50% methanol / deionized water (v/v) (approx. 15 ml, flow 0.7 ml/min). Before use, the system should be equilibrated with approx. 30 ml Eluent.

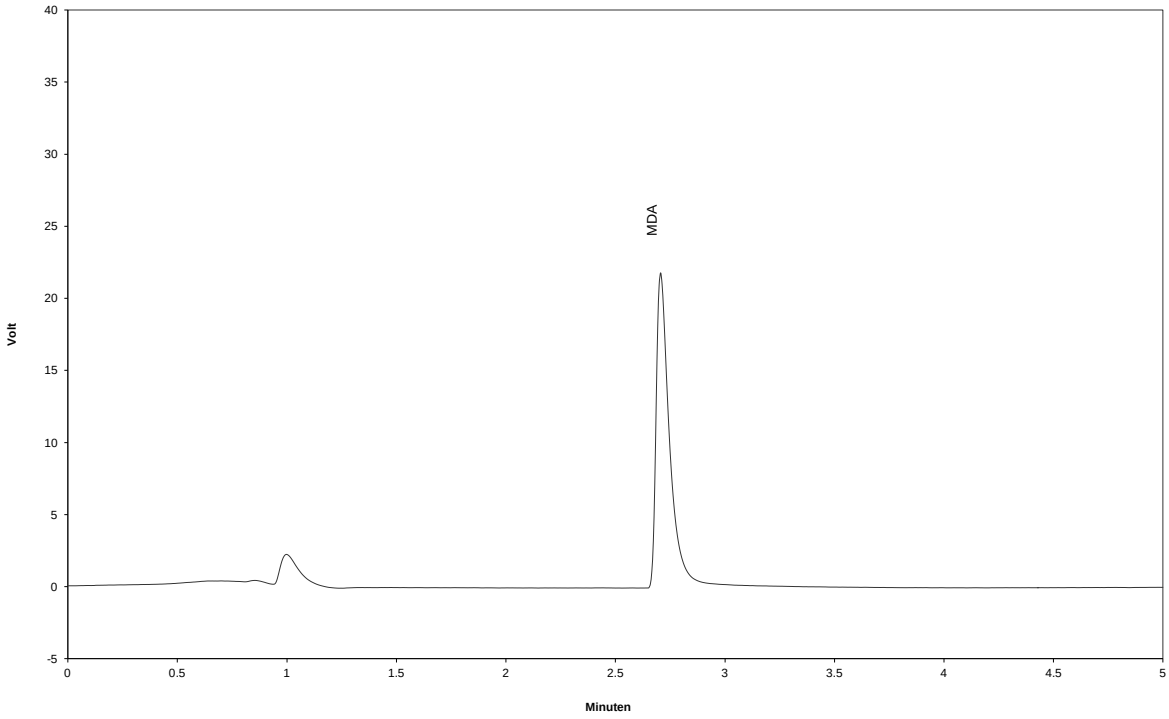
11. Calculation of the analytical results

Calculation

$$\text{Conc. sample} = \frac{\text{peak height patient} * \text{conc. calibrator}}{\text{peak height calibrator}}$$

Please take into account that the calculated peak heights of calibrator and samples are the peak heights from which the blank value has been subtracted.

Typical chromatogram



10. Internal Quality Control

Reference values

EDTA plasma: **< 1 µmol/l**

We recommend that each laboratory 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:	9.0 % (0.86 µmol/l)	[n = 6]
	6.4 % (2.55 µmol/l)	[n = 6]
Inter-Assay CV:	10.9 % (0.89 µmol/l)	[n = 6]
	7.5 % (2.50 µmol/l)	[n = 6]

Linearity

up to 50 µmol/l

Detection limit

0.01 µmol/l

Recovery

95.7 %

12. Limitations of the method

- We recommend not to measure strong hemolytic and lipemic samples, because they might show pathological concentrations.
- Whole blood is not suited for the Malondialdehyde (MDA) HPLC Assay kit.

13. Disposal

The mobile phase (ELU), derivatisation solution (DERIVAT) and the reaction solution (REAL) must be disposed as non-halogenated solvent.

Please refer to the appropriate national guidelines.

14. Troubleshooting

Problem	Possible reason	Solution
No signal	No or defect connection to evaluation system	Check signal cord and connection
	Detector lamp is altered	Change lamp
No peaks	Injector is congested	Check Injector
Double peaks	Dead volume in fittings and / or column	Renew fittings and / or column
Contaminating peaks	Injector dirty	Clean injector
	Contamination at the head of the column	Change direction of the column and rinse for 30 min at low flow rate (0.2 ml/min) with mobile phase
	Air in the system	Degas pump
	Autosampler vials contaminated	Use new vials or clean them with methanol
Broad peaks, tailing	Precolumn / column exhausted	Use new precolumn / column
Variable retention times	Drift in temperature	Use a column oven
	Pump delivers imprecise	Check pump, degas the system
	System is not in steady state yet	Rinse system mobile phase for 15 min
Baseline is drifting	Detector lamp did not reach working temperature yet	Wait
	Detector lamp is too old	Renew lamp
Continue baseline is drifting	System is not in steady state yet	Rinse system mobile phase for 15 min
	Pump delivers imprecise	Check pump, degas the system
Baseline is not smooth	Pump delivers imprecise	Check pump, degas the system
	Detector flowcell is dirty	Clean flow cell

15. Literature references

- Draper H.H., Hadley M. (1990). A review of recent studies on the metabolism of exogenous and endogenous malondialdehyde. Xenobiotika 20; 9; 901-907.

- Griesmacher A. et al. (1995). Enhanced serum levels of thiobarbitric-acid-reactive substances in diabetes mellitus. Am J Med 98; 469-475.

- Valenzuela A (1990). The biological significance of malondialdehyde determination in the assessment of tissue oxidative stress. Life sciences 48; 301-309.

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.