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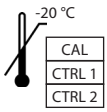
Manual

Malondialdehyde HPLC Kit

For the determination of malondialdehyde in plasma, serum and urine

Valid from 2022-09-05

REF **KC1900**



IVD CE

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1. INTENDED USE

The Immundiagnostik AG assay is intended for the quantitative determination of malondialdehyde in plasma, serum and urine. This assay is designed for *in vitro* diagnostic use only.

2. SUMMARY AND EXPLANATION OF THE TEST

In the last years the damage effects of lipid peroxidation products were studied intensively. These are formed when free radicals will overcome the radical-trapping-mechanisms of the body and reacting with unsaturated fatty acids.

The reaction of polyunsaturated fatty acids (PUFA´s) with activated oxygen species results in lipidhydroperoxides (primary lipid peroxidation products) which were degraded to secundary lipid peroxidation products like alkanes (e.g. ethane and pentane), aliphatic aldehydes (e.g. malondialdehyde [MDA] and 4-hydroxynonenale [4-HNE]).

Primary and secondary lipid peroxidation products have an influence on a lot of molecules responsible for correct cell function.

So lipidhydroperoxides easily pass the nuclear membrane and can react with nucleic acids. Also proteins can be attacked on their thiol groups changing their stereochemistry and function.

Moreover lipidhydroperoxides interfere with chemical and physical properties of the cell membrane. The fluidity decreases and rigidity increases. The so influenced cell membrane can't maintain their barrier function and intracellular potassium ions leak out as well as intracellular enzymes are lost. If erythrocytes are afflicted haemolysis takes place. In this case haemoglobin can initiate or propagate the lipid peroxidation.

Secondary lipid peroxidation products like MDA or 4-HNE can react with DNA as well, in particular with the bases guanin and adenin. These DNA aberrations lead to erroneous transcriptions and therewith to altered gene products. Peptide bonds are broken through the impact of MDA in proteins. Aldehydes react with amino groups of proteins building Schiff-bases, elementary disturbing correct function of proteins.

All these toxic features of oxidised fatty acids are discussed in the pathogeneses of many diseases and dysfunction of organs. In particular, arteriosclerosis, tumor diseases, rheumatic diseases and reperfusion damage of organs after ischämic processes.

3. PRINCIPLE OF THE TEST

The first step in determining malondialdehyde is a sample preparation with derivatisation. The derivatisation reagent transforms malondialdehyde into a fluorescent product. Afterwards the pH is optimised through to addition of a reaction solution. 20 µl of the supernatant are injected into the HPLC system.

The separation via HPLC follows an isocratic method at 30 °C using a reversed phase column. One run lasts 4 minutes. The chromatograms are recorded by a fluorescence detector. The quantification is performed with the delivered plasma calibrator; the concentration is calculated via integration of the peak heights by the external standard method.

Summary

Besides many other parameters the advantage of HPLC method lies in the simultaneous handling of many analytes in a single test. The HPLC system enables even laboratories without experience in high performance liquid chromatography to use this technique for clinical routine determination in a quick and precise manner. Unlike immuno assays with up to six calibrators per test, a one-point calibration is mostly sufficient to calibrate the test system. It is possible to automate the sample application and calculation of the results so that even higher numbers of samples can be handled nearly without control.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KC 1900	MOPHA	Mobile phase	1 000 ml
	CAL	Calibrator; lyophilised (see specification data sheet for concentration)	4 x
	DER	Derivatisation solution	100 ml
	REABUF	Reaction solution	50 ml
	CTRL 1	Control 1; lyophilised	4 x
	CTRL 2	Control 2; lyophilised	4 x

For reorders of single components, use the catalogue number followed by the label as product number.

The HPLC column (KC1900RP), can be ordered separately from Immundiagnostik. To extend the lifetime of your HPLC column, pre-columns (KC1900VS) are highly recom-

mended. These and also the pre-column holders (KC1900VH) can also be ordered from Immundiagnostik. In addition to the complete kits, all components can also be ordered separately. Please ask for our single component price list.

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Vortex
- 1.5 ml reaction tubes (Eppendorf)
- Centrifuge
- Various pipettes
- HPLC with Fluorescence-detector
- Reversed phase C18-column - Bischoff Prontosil Eurobond C_{18} 5 µm, 125 x 4 mm
- Water bath or heating block for heating at 95 °C

  * Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

6. PREPARATION AND STORAGE OF REAGENTS

- Reconstitute the **calibrator** (CAL) in **250 µl** ultrapure water. Take aliquots and store at -20 °C. Reconstituted calibrator is stable for at least 2 weeks at -20 °C. Avoid repeated freeze-thaw circles. The concentration of malondialdehyde might have minor changes from lot to lot.
- Reconstitute **controls** (CTRL1, CTRL2) in **250 µl** ultrapure water. **Storage** details are given in the **specification data sheet**.
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

7. SPECIMEN COLLECTION AND PREPARATION

Plasma, serum and urine are suited for this test system. After venipuncture the sample should be stored at 2–8 °C immediately. Samples are stable for at least 24 h at 2–8 °C and 2 weeks at -20 °C.

8. ASSAY PROCEDURE

Sample preparation

Important: work with a water blank as a fluorescent compound is produced from the derivatisation solution (DER) that has the same retention time as the MDA-derivatisation product. The obtained blank value must be subtracted from all preparations.

Pipet each **20 µl ultrapure water** (blank)**, sample**, **calibrator** (CAL) **or control 1 or 2** (CTRL1, CTRL2) into a 1.5 ml reaction tube.
Add each **1 ml derivatisation solution** (DER) and vortex for **15 seconds**.
Incubate for **60 min** at 95 °C.

Keep incubation time and temperature constant as only at these conditions the given MDA-concentrations for calibrator (CAL) and controls (CTRL1, CTRL2) are valid.

It is possible that the lids of the reaction tube open during incubation. This can be avoided by putting a weight on the reaction tubes.

Cool down the solution (**min. 15 min at 2–8 °C**) and centrifuge for 5 min.
Take **500 µl** supernatant + **500 µl** reaction solution (REABUF) and mix thoroughly on a vortex mixer.
Inject **20 µl** of the mixture into the HPLC system.

The derivatised sample is stable at 2–8 °C for at least 4 days and at room temperature for at least 12 hours.

Chromatographic conditions

Important:	No recirculation of the eluent is allowed for this test-system		
Column material:	Bischoff Prontosil Eurobond, 5 µm		
Column dimension:	125 mm x 4 mm		
Flow rate:	0.8–1.2 ml/min		
Detection:	Fluorescence:	Exitation	515 nm
		Emission	553 nm
Temperature:	30 °C		
Injection volume:	20 µl		
Running time:	4 min		

It is recommended that a guard column is used to extend column life.

9. TREATMENT OF THE COLUMN

After the analysis the column should be flushed with 30 ml ultrapure water (1 ml/min) and stored in 50 % methanol in aqua bidest (ca. 30 ml, flow 0.7 ml/min). Before use, the system should be equilibrated with ca. **30 ml MOPHA** (mobile phase).

Important: Do not recirculate the MOPHA (mobile phase) in this test system.

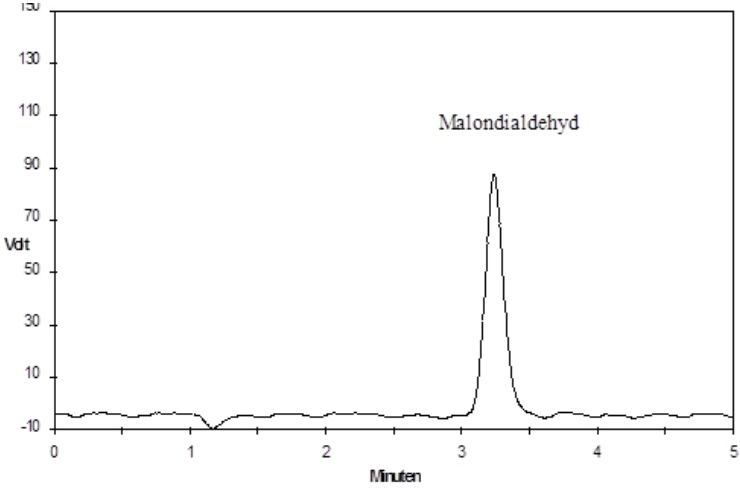
10. RESULTS

Calculation

Take into account that the mentioned heights of calibrator and patient are the heights from which the blank value has been subtracted.

$$\text{Concentration sample} = \frac{\text{Peak height sample x Concentration of the calibrator}}{\text{Peak height calibrator}}$$

Typical chromatogram



11. LIMITATIONS

Do not use whole blood.

Strong haemolytic and lipaemic samples often show pathological concentrations. Do not measure such samples.

12. QUALITY CONTROL

Expected values

Serum, heparin plasma: **1.97 ± 0.41 µmol/l**

EDTA plasma: **0.61 ± 0.24 µmol/l**

It is recommended that each laboratory should establish its own normal range. Above mentioned values are only for orientation and may vary from other published data.

Controls

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

13. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n = 12

The repeatability was assessed with one control sample under **constant** parameters (same operator, measurement system, day and kit lot).

Mean value [µmol/l]	CV [%]
5.98	4.0

Reproducibility (Inter-Assay); n = 21

The reproducibility was assessed with 2 control samples under **varying** parameters (different operators, measurement systems, days and kit lots).

Sample	Mean value [µmol/l]	CV [%]
1	2.58	9.3
2	5.51	9.7

Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, three serum samples and three control samples were spiked with known amounts of Malondialdehyde three times. The mean values are shown in the table below.

The recovery for Malondialdehyde was found between 96.38 bis 102.95 %.

Sample	Spike [µmol/l]	Expected [µmol/l]	Obtained [µmol/l]	Recovery [%]
3.62	5	8.62	8.79	101.92
4.66	5	9.66	9.79	101.38
3.16	5	8.16	8.39	102.76
2.50	5	7.50	7.23	96.38
4.09	5	9.09	9.17	100.92
1.95	5	6.95	7.16	102.95

Lower detection limit

The limit of detection (LoD) is defined as 3 times the background noise. It is calculated by the formula below:

$$\frac{(3 \text{ x peak height background noise}) \text{ x concentration calibrator [µmol/l]}}{\text{Peak height calibrator}} = \text{LoD [µmol/l]}$$

LoD Malondialdehyde: 0.064 µmol/l

The limit of quantitation (LoQ) is defined as 10 times the background noise. It is calculated by the formula below:

$$\frac{(10 \text{ x peak height background noise}) \text{ x concentration calibrator [µmol/l]}}{\text{Peak height calibrator}} = \text{LoQ [µmol/l]}$$

LoQ Malondialdehyde: 0.213 µmol/l

Upper detection limit & linearity

The upper limit of detection states up to which concentration a method results in a linear signal. Therefore, 6 samples with known concentrations were measured two times.

Between the range from 2.5 to 105 µmol/l non-linearity was found below 20 %.

Sample [µmol/l]	Signal height Experiment 1	Signal height Experiment 2
2.5	8 805	10 862
10	34 561	33 645
15	56 909	54 170
25	102 935	97 879
55	249 409	236 687
105	489 665	474 167

It should be noted that the detection limits depend on the instrument as well as on the application.

Analytical specificity

There were found no interferences to other blood components.

14. DISPOSAL

The mobile phase (MOPHA), derivatisation solution (DER) and reaction solution (REABUF) must be disposed as non-halogenated solvent.

Please refer to the appropriate national guidelines.

15. 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

Problem	Possible reason	Solution
No peaks	Injector is congested	Check Injector
Doublepeaks	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 yet reach working temperature	Wait
	Detector lamp is too old	Renew lamp
	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 flow cell is dirty	Clean flow cell

16. PRECAUTIONS

- Control samples should be analysed with each run.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.
- The assay should always be performed according to the enclosed manual.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- The test components contain organic solvents. Contact with skin or mucous membranes has to be avoided.
- The derivatisation solution (DER) contains acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.
- As a precaution, it is recommended that the human material used is always considered potentially infectious.

17. GENERAL NOTES ON THE TEST

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Do not mix plugs and caps from different reagents.

- Do not interchange different lot numbers of any kit component within the same assay.
- Quality control guidelines should be observed.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

18. REFERENCES

1. Draper H.H., Hadley M. (1990). A review of recent studies on the metabolism of exogenous and endogenous malondialdehyde. *xenobiotika* **20**; 9; 901-907.
2. Griesmacher A. et al. (1995). Enhanced serum levels of thiobarbituric-acid-reactive substances in diabetes mellitus. *Am J Med* **98**; 469-475.
3. Valenzuela A (1990). The biological significance of malondialdehyde determination in the assessment of tissue oxidative stress. *Life sciences* **48**; 301-309

Used symbols:

	Temperature limitation		Catalogue number
	In Vitro Diagnostic Medical Device		To be used with
	Manufacturer		Contains sufficient for <n> tests
	Lot number		Use by
	Contains plasma derivatives or human blood		Consult instructions for use
	Consult specification data sheet		Do not re-use
	Unique Device Identification		Contains material of animal origin
	Medicinal substance		Contains material of human origin