Malondialdehyde HPLC Kit

For the determination of Malondialdehyde in plasma, serum and urine

Valid from 31.08.2010
1. INTENDED USE

The Immundiagnostik 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 lipid hydroperoxides (primary lipid peroxidation products) which were degraded to secondary 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 oxidized 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 optimized 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**

<table>
<thead>
<tr>
<th>Catalogue No</th>
<th>Content</th>
<th>Kit Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>KC 1900LM</td>
<td>MOPHA</td>
<td>Mobile phase</td>
<td>1000 ml</td>
</tr>
<tr>
<td>KC 1900KA</td>
<td>CAL</td>
<td>Calibrator, lyophilized</td>
<td>5 vials</td>
</tr>
<tr>
<td>KC 1900DL</td>
<td>DER</td>
<td>Derivatisation solution</td>
<td>100 ml</td>
</tr>
<tr>
<td>KC 1900RL</td>
<td>REABUF</td>
<td>Reaction solution</td>
<td>50 ml</td>
</tr>
<tr>
<td>KC1900KO</td>
<td>CTRL1</td>
<td>Control 1 and 2, 250 μl lyophilized</td>
<td>2 x 3 vials</td>
</tr>
</tbody>
</table>

HPLC column (KC 1900RP) as well as individual components can be ordered separately from Immundiagnostik. Please ask for the price list of the individual components.
5. MATERIAL REQUIRED BUT NOT SUPPLIED

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

6. PREPARATION AND STORAGE OF REAGENTS

- Reconstitute the calibrator (CAL) in 250 μl aqua bidist. 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 aqua bidest.

- All other test reagents are stable at 2-8 °C, up to the date of expiry stated on the label.

7. PRECAUTIONS

- For in vitro diagnostic use only.

- This product contain human source material which was tested and found to be non-reactive to HBsAg, anti-HIV-1/2, and anti-HCV. Since no method can offer complete assurance that hepatitis B virus, HIV-1/2, HVC or other infectious agents are absent, these reagents should be handled as if potentially infectious.

- 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.

- Reagents should not be used beyond the expiration date shown on kit label.
8. 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.

9. ASSAY PROCEDURE

Procedural notes

- Quality control guidelines should be observed.
- 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 the enclosed manual.
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 in a 1.5 ml reaction tube (e.g. Eppendorf):

- **20 μl** water, patient sample, calibrator (CAL) or controls (CTRL1, CTRL2) +

- **1 ml** derivatisation solution (DER) and mix for **15 seconds** on a vortex mixer.

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.

Cool down the solution (**min. 15 min at 2-8 °C**) and centrifuge for 5 min.

Take **500 μl** supernatant and **500 μl** reaction solution (REABUF) and mix thoroughly on a vortex mixer.

Inject **20 μl** of the mixture into the HPLC system.

The derivatized 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.
10. TREATMENT OF THE COLUMN

After the analysis the column should be flushed with 30 ml aqua bidist (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 re-circulate the MOPHA (mobile phase) in this test system.

11. RESULTS

Calculation

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

\[
\text{Concentration sample} = \frac{\text{Peak height sample} \times \text{Concentration of the calibrator}}{\text{Peak height calibrator}}
\]

Typical chromatogram
12. LIMITATIONS

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

13. QUALITY CONTROL

Expected values
Serum, heparin plasma: \(1.97 \pm 0.41 \text{ μmol/l}\)
EDTA plasma: \(0.61 \pm 0.24 \text{ μmol/l}\)

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 analyzed 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.
14. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

**Intra-Assay CV:**
- 6.1 % (0.48 μmol/l) [n = 12]
- 4.8 % (2.06 μmol/l) [n = 12]

**Inter-Assay CV:**
- 6.9 % (0.46 μmol/l) [n = 12]
- 5.7 % (2.13 μmol/l) [n = 12]

Linearity

up to 100 μmol/l

Detection limit

0.15 μmol/l

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


18. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- The test components contain organic solvents. Contact with skin or mucous membranes has to be avoided.
- All reagents in the test package are for research use only.
- The reagents should not be used after the date of expiry stated on the label.
- Do not interchange different lot numbers of any kit component within the same assay.
- Quality control guidelines should be observed.
- 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.