Vitamin A/E HPLC Kit

Zur Bestimmung von Vitamin A/E in Plasma und Serum

For the determination of vitamin A/E in plasma and serum

EU: IVD / CE

Gültig ab / Valid from 04.08.2014

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1. INTENDED USE
This Immundiagnostik assay is intended for the quantitative determination of vitamin A/E in serum and plasma. This assay is designed for in vitro diagnostic use only.

2. INTRODUCTION
Vitamin A (Retinol) and vitamin E (tocopherol) are fat-soluble vitamins, which can be stored for longer periods in the adipose tissue. Both, lack and excess, can express in complaints.
Vitamin A is essential for the visual process and recovers the skin and mucosa. A lack of vitamin A will reduce the visual power up to total blindness. An excess of vitamin A could cause headache, damage of the skin, liver disease, painful alteration in the skeleton or foetal damage.
Vitamin E (tocopherol) is an antioxidant and protects unsaturated fatty acids against oxidation. It also protects the cells of the body by catching radicals.
A lack of vitamin E in animal experiments demonstrates diseases of muscle, nervous system, heart, liver and reproduction system. These symptoms were not observed in humans. Vitamin E can be stored in the adipose tissue in large amounts. A lack can be caused by a malfunction in digestion or resorption of fatty acids.

3. PRINCIPLE OF THE TEST
The first step in the determination of vitamin A and E includes the sample preparation. In the first step an internal standard solution is added. During the precipitation higher molecular substances are removed. After centrifugation the supernatant is used for injection into the HPLC system.
The separation via HPLC follows an isocratic method at 30 °C using a “reversed phase” column; one run lasts 15 minutes. The detection is performed by an UV detector at two different wavelengths (Vitamin A: 325 nm; Vitamin E: 300 nm). The quantification is performed with the delivered standard solution; the concentration is calculated via integration of the peak areas in the internal standard calibration mode.

Summary:
The application of vitamin A and E for HPLC allows the determination of both vitamins in an easy, fast, and precise method. The kit includes all reagents in ready to use form for preparation and separation of the samples with exception of the columns.
As many other parameters, the advantage of HPLC analysis is the simultaneous handling of many analytes in a single test. The HPLC complete system enables even laboratories without experience in high performance liquid chromatography to use
this technique for clinical chemical routines quickly and precisely. Mostly a one-point calibration is sufficient for calibrating the test system – unlike immuno assays with up to 6 calibrators per test. It is possible to automate the sample application and calculation of the results so that even higher number 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>KC1600LM</td>
<td>MOPHA</td>
<td>Mobile phase</td>
<td>1000 ml</td>
</tr>
<tr>
<td>KC1600ST</td>
<td>STD</td>
<td>Standard (conc. is given on the label)</td>
<td>10 ml</td>
</tr>
<tr>
<td>KC1600IS</td>
<td>INTSTD</td>
<td>Internal Standard</td>
<td>5 ml</td>
</tr>
<tr>
<td>KC1600FR</td>
<td>PREC</td>
<td>Precipitation reagent</td>
<td>50 ml</td>
</tr>
<tr>
<td>KC1600VL</td>
<td>DIL</td>
<td>Dilution solution</td>
<td>10 ml</td>
</tr>
<tr>
<td>KC1600KO</td>
<td>CTRL 1</td>
<td>Control 1 and 2; 600 µl lyophilized</td>
<td>2 x 3 vials</td>
</tr>
</tbody>
</table>

The HPLC column (KC1600RP) 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

- Ultra pure water*
- 1.5 ml reaction tubes (Eppendorf)
- Centrifuge
- Various pipettes
- HPLC with UV-detector
- Reversed phase C18-column

* Immundiagnostik AG recommends the use of Ultra Pure 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 CTRL 1 and CTRL 2 (control 1 and 2) in 600 µl ultra pure water
- All other reagents are ready to use and are delivered in soluble form.
- All reagents are stable at 20–25°C; STD (standard), CTRL 1 and CTRL 2 (control 1 and 2) and INTSTD (internal standard) at -20°C up to the date of expiry (see label of the test package).

7. PRECAUTIONS

- For in vitro diagnostic use only.
- This product contains 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.
- Reagents should not be used beyond the expiration date shown on kit label.

8. SPECIMEN COLLECTION AND PREPARATION

Serum and EDTA plasma are suited as samples. The sample is light and temperature sensitive; therefore samples have to be protected from light and cooled and centrifuged immediately. The samples are stable in the dark at 2–8°C for 12 h (vitamin A) and 48 h (vitamin E). At -20°C, vitamin A is stable for 1 month, vitamin E for 3 months.

9. ASSAY PROCEDURE

Procedural notes

- The quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure, that are not coordinated with the producer, may influence the test results. Immundiagnostik can therefore not be held reliable for any damage resulting from this.
- The assay should always be performed due to the manual which is given in the kit.
**Sample and standard preparation**

Pipet into 1.5 ml reaction tubes:

<table>
<thead>
<tr>
<th></th>
<th>Standard:</th>
<th>Samples, CTRL 1 and CTRL 2 (control 1 and 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>250 µl STD (standard)</td>
<td>250 µl sample or CTRL 1, CTRL 2</td>
</tr>
<tr>
<td>2.</td>
<td>+ 50 µl INTSTD (internal standard)</td>
<td>+ 50 µl INTSTD (internal standard)</td>
</tr>
<tr>
<td>3.</td>
<td>+ 250 µl DIL (dilution solution)</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>+ 250 µl PREC (precipitation solution)</td>
<td>+ 500 µl PREC (precipitation solution)</td>
</tr>
<tr>
<td>5.</td>
<td>Mix well. Leave the tubes for <strong>30 minutes</strong> at 2–8 °C and centrifuge afterwards at <strong>10 000 g</strong> for <strong>10 minutes</strong>.</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Inject <strong>100 µl</strong> of the supernatant for chromatography into the HPLC-system.</td>
<td></td>
</tr>
</tbody>
</table>

**Chromatographic conditions**

- **Column material:** Nucleosil C18; 10 µm
- **Column dimension:** 125 mm × 4 mm
- **Flow rate:** 0,8–1,2 ml/min
- **UV detection:**
  - Vitamin A: 325 nm
  - Vitamin E: 300 nm
- **Injection volume:** 100 µl
- **Running time:** 15 min
- **Temperature:** 30 °C

The wavelength should be switched after 7 min.

To avoid contamination of the next run, MOPHA (mobile phase) must be used to wash the auto sampler.

Immundiagnostik recommends to use a guard-column to enlarge lifetime of the
column.

10. TREATMENT OF THE COLUMN
After analysis, the column could be left in MOPHA (mobile phase). Before use, the system should be equilibrated with ca. 30 ml MOPHA (mobile phase).

11. RESULTS

Calculation
\[
\frac{\text{Peak height sample} \times \text{concentration calibrator}}{\text{Peak height internal standard in the sample}} \times F = \text{concentration of the calibrator}
\]
\[
F = \frac{\text{Peak height internal standard in the calibrator}}{\text{Peak height calibrator}}
\]

Typical chromatogram

12. LIMITATIONS
Hemolytic and lipemic samples should not be measured.
13. QUALITY CONTROL

**Expected values**

- Vitamin A: 200–800 µg/l
- Vitamin E: 3–14 mg/l

We recommend each laboratory to establish an own reference range as reference ranges depend on the chosen subjects. The above mentioned values are meant to be a guideline only and can deviate from other published data.

**Controls**

Control samples or serum pools should be analyzed with each run of calibrators and patient samples. Results generated from the analysis of the control samples should be evaluated for acceptability using appropriate statistical methods. In assays in which one or more of the quality control sample values lie outside the acceptable limits, the results for the patient sample may not be valid.

14. PERFORMANCE CHARACTERISTICS

**Precision and reproducibility**

**Intra-Assay VK**

- Vitamin A: 1.9 % (0.55 mg/l) \( [n = 6] \)
- Vitamin A: 1.2 % (1.18 mg/l) \( [n = 6] \)
- Vitamin E: 1.5 % (9.0 mg/l) \( [n = 6] \)
- Vitamin E: 1.1 % (14.9 mg/l) \( [n = 6] \)

**Inter-Assay VK**

- Vitamin A: 4.9 % (0.6 mg/l) \( [n = 8] \)
- Vitamin A: 3.1 % (1.0 mg/l) \( [n = 8] \)
- Vitamin E: 4.6 % (8.3 mg/l) \( [n = 8] \)
- Vitamin E: 4.7 % (24 mg/l) \( [n = 8] \)

**Linearity**

- Vitamin A: up to 10 mg/l
- Vitamin E: up to 50 mg/l
Detection limit
Vitamin A: 0.05 mg/l
Vitamin E: 1 mg/l

Recovery
Vitamin A: 98.8 %
Vitamin E: 101 %

15. DISPOSAL
MOPHA (mobile phase), PREC (precipitation reagent), INTSTD (internal standard) and STD (standard) must be disposed as non-halogenated solvent.
Please refer to the appropriate national guidelines.

16. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible reasons</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</td>
<td>Injector dirty</td>
<td>Clean injector</td>
</tr>
<tr>
<td>peaks</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></td>
<td>Autosampler vials contaminated</td>
<td>Use new vials or clean them with methanol</td>
</tr>
<tr>
<td>Problem</td>
<td>Possible reasons</td>
<td>Solution</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------</td>
<td>----------</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 calm</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 to be used for *in vitro* diagnostic use only.
- The reagents should not be used after the date of expiry stated on the label.
- Single components with different lot numbers should not be mixed or exchanged.
• The guidelines for medical laboratories should be observed.
• Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure, that are not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held reliable for any damage resulting from this.

**Used symbols:**

- **Temperature limitation**
- **Catalogue Number**
- **In Vitro Diagnostic Medical Device**
- **Contains sufficient for \(<n>\) tests**
- **Manufacturer**
- **Use by**
- **Lot number**