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Manual

Vitamin A/E HPLC Kit

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

Valid from 2022-09-05

REF **KC1600**

Σ 100

IVD

CE

+8°C
+2°C

-20°C

STD
INT STD
CTRL 1
CTRL 2

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1. INTENDED USE

This HPLC application 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/heights 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 reagents in ready to use form for preparation and separation of the samples with exception of the columns.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KC1600	MOPHA	Mobile phase (important: do not recirculate)	1 000 ml
	STD	Standard; ready-to-use (see label for concentration)	10 ml
	INT STD	Internal Standard; ready-to-use	5 ml
	PREC	Precipitation reagent; ready-to-use	50 ml
	DIL	Dilution solution	10 ml
	CTRL 1	Control 1; lyophilised	4 x
	CTRL 2	Control 2; lyophilised	4 x

The HPLC column (KC1600RP), can be ordered separately from Immundiagnostik. To extend the lifetime of your HPLC column, pre-columns (KC1600VS) are highly recommended. These and also the pre-column holders (KC1600VH) 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

- Ultra pure water*
- 1.5 ml reaction tubes (Eppendorf)
- Centrifuge
- Various pipettes (100 µl, 1 000 µl)
- 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

- The lyophilised controls 1 and 2 (**CTRL 1 and CTRL 2**) are stable at -20 °C until the expiry date stated on the label. Before use, they have to be reconstituted with each 600 µl ultra pure water. The concentration of vitamin A/E slightly changes from lot to lot, the exact concentration is stated on the specification.

- The standard (**STD**) is stable at -20 °C until the expiry date stated on the label. The concentration of vitamin A/E slightly changes from lot to lot, the exact concentration is stated on the label.
- The internal standard (**INT STD**) is stable at -20 °C until the expiry date stated on the label.
- All other test reagents are stable until the expiry date (see label of test package) when stored at 2–8 °C.

7. 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.

8. ASSAY PROCEDURE

Sample and standard preparation

Pipet into 1.5 ml reaction tubes:

	Standard:	**Samples and controls**
1.	250 µl standard (STD)	250 µl sample or controls (CTRL1 and 2)
2.	+ 50 µl internal standard (INT STD)	+ 50 µl internal standard (INT STD)
3.	+ 250 µl dilution solution (DIL)	
4.	+ 250 µl precipitation reagent (PREC)	+ 500 µl precipitation reagent (PREC)
5.	Mix well. Leave the tubes for 30 minutes at 2–8 °C and centrifuge afterwards at 10 000 *g* for 10 minutes.	
6.	Inject 100 µl of the supernatant for chromatography into the HPLC-system.	

Chromatographic conditions

Column material:	Nucleosil C18; 10 µm
Column dimension:	125 mm × 4 mm
Flow rate:	0.8–1.2 ml/min
	Please refer to the quality certificate of the column
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, mobile phase (**MOPHA**) must be used to wash the auto sampler.

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

9. TREATMENT OF THE COLUMN

After analysis, the column could be left in mobile phase (**MOPHA**). Before use, the system should be equilibrated with ca. 30 ml mobile phase (**MOPHA**), first without, then with the column.

10. RESULTS

Calculation

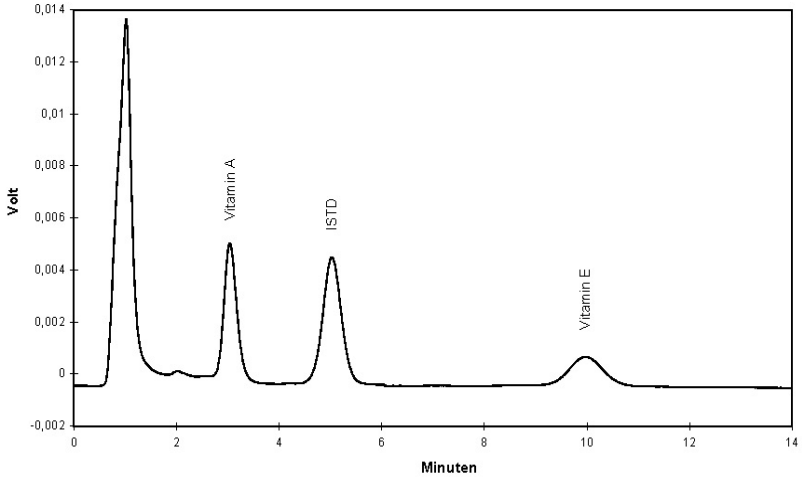
$$\frac{\text{Peak height sample} \times \text{concentration standard}^*}{\text{Peak height internal standard in the sample}} \times F = \text{concentration of the sample}$$

$$F = \frac{\text{Peak height internal standard in the standard}}{\text{Peak height standard}}$$

* see label

Tip: Alternatively, the peak area instead of the peak height can be used for quantification.

Typical chromatogram



11. LIMITATIONS

Hemolytic and lipemic samples should not be measured.

12. 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.

13. 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 %

14. DISPOSAL

Mobile phase (**MOPHA**), precipitation reagent (**PREC**), internal standard (**INT STD**) and standard (**STD**) must be disposed as non-halogenated solvent.

Please refer to the appropriate national guidelines.

15. TROUBLESHOOTING

Problem	Possible reasons	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

Problem	Possible reasons	Solution
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
	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 calm	Pump delivers imprecise	Check pump, degas the system
	Detector flow cell is dirty	Clean flow cell

16. PRECAUTIONS

- 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.
- Reagents should not be used beyond the expiration date shown on kit label.
- As a precaution, it is recommended that the human material used is always considered potentially infectious.
- The test components contain organic solvents. Contact with skin or mucous membranes has to be avoided.

17. 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.
- 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.
- The quality control guidelines should be observed.
- The assay should always be performed due to the manual which is given in the kit.
- 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.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.

18. REFERENCES

1. Sushil K.J., Mc Coy B., Wise R. (1994). Vitamin E and the hypercoagulability of neonatal blood. *Clin Cim Acta* **225**; 97-103.
2. Comstock G.W., Alberg A.J., Helzlsouer K.J. (1993). Reported effects of long-term freezer storage on concentrations of retinol, β-carotene, and α-tocopherol in serum or plasma summarised. *Clin Chem* **39**/6; 1075-1078

Used symbols:


Temperature limitation

In Vitro Diagnostic Medical Device


Manufacturer

Lot number


Contains plasma derivatives or human blood

Consult specification data sheet

Unique Device Identification


Medicinal substance

Catalogue number

To be used with

Contains sufficient for <n> tests


Use by


Consult instructions for use


Do not re-use

Contains material of animal origin

Contains material of human origin