Manual

For professional use only

Vitamin A/E HPLC Kit

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

Valid from 2023-08-28

REF **KCR1600**

Σ 100

RUO

+2 °C +8 °C

-20 °C

CAL
INTSTD
CTRL1
CTRL2

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Safety information

These accessories are to be used exclusively in accordance with the enclosed instructions for use. Important safety information for this product can be found in chapter 16.

Table of Contents

1. INTENDED PURPOSE

This HPLC application is intended for the quantitative determination of vitamin A/E in serum and plasma. This assay is for research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Vitamin A and E belong to the fat-soluble vitamins and can be stored in the fatty tissue over a longer period of time. Both an over- and an undersupply of vitamins A and E can manifest themselves in complaints.

Vitamin A (retinol) is essential for the visual process and keeps the skin and mucous membranes healthy. A deficiency of vitamin A can therefore have an impact on vision, especially during transitions from light to dark. A severe vitamin A deficiency can lead to blindness. Too high doses however, can also have a pathological effect and cause headaches, skin changes, liver damage, painful skeletal changes and damage to the foetus.

Vitamin E (tocopherol) is a natural antioxidant that protects unsaturated fatty acids from oxidation. It intercepts the radicals before they can have a destructive effect on the cell. A deficiency of vitamin E showed damage to the musculature, nervous system and heart, liver and reproduction in animal experiments. In humans such effects have not been observed. Vitamin E can be stored in large quantities in fatty tissue. An undersupply can occur due to impaired fat digestion or absorption.

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 8 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 calibrator; 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
KCR1600	MOPHA	Mobile phase, ready-to-use (important: do not recirculate)	1 000 ml
	INTSTD	Internal standard, ready-to-use	5 ml
	PREC	Precipitation reagent, ready-to-use	50 ml
	CAL	Calibrator, lyophilised	4 x
	CTRL1	Control 1, lyophilised	4 x
	CTRL2	Control 2, lyophilised	4 x
KR 0005.15	RECSOL	Reconstitution solution	15 ml

The HPLC column (KCR1600RP), can be ordered separately from Immundiagnostik AG. To extend the lifetime of your HPLC column, pre-columns (KCR1600VS) are highly recommended. These and also the pre-column holders (KCR1600VH) can also be ordered from Immundiagnostik AG.

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

- 1.5 ml reaction tubes (Eppendorf)
- Centrifuge
- Various pipettes (100 µl, 1 000 µl)
- Vortex
- HPLC system with UV-detector
- Reversed phase C18-column

6. PREPARATION AND STORAGE OF REAGENTS

- The lyophilised controls (**CTRL1 and CTRL2**) are stable at -20 °C until the expiry date stated on the label. Before use, they have to be reconstituted each with x µl reconstitution solution (**RECSOL**) (x = please see product specification). The concentration of vitamin A/E slightly changes from lot to lot, the exact concentration is stated in the product specification.

- The lyophilised calibrator (**CAL**) is stable at -20 °C until the expiry date stated on the label. Before use, it has to be reconstituted with x µl reconstitution solution (**RECSOL**) (x = please see product specification). The concentration of vitamin A/E slightly changes from lot to lot, the exact concentration is stated in the product specification.
- The internal standard (**INTSTD**) 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 3 days (vitamin E). Vitamin A is stable for 1 month, vitamin E for at least 3 months when stored at -20 °C.

8. ASSAY PROCEDURE

Sample and standard preparation

Pipet into 1.5 ml reaction tubes:

1.	**250 µl** sample, calibrator (**CAL**) and controls (**CTRL1** and **CTRL2**)
2.	Add **50 µl** internal standard (**INTSTD**)
4.	Add **500 µl** precipitation reagent (**PREC**)
5.	Mix well, incubate for **30 minutes** at **2–8 °C** and then centrifuge at **10 000 *g*** for **5 minutes**.
6.	Inject **100 µl** of the supernatant for chromatography into the HPLC system.

Chromatographic conditions

Column material:	Nucleosil C18; 10 µm alternatively: VDSpher PUR C18 M-E; 10 µm
Column dimension:	125 mm × 4 mm
Flow rate:	2.0 ml/min
UV detection:	Vitamin A: 325 nm
	Vitamin E: 300 nm
Injection volume:	100 µl
Running time:	8 min
Temperature:	30 °C

Switch the wavelength after approx. 4 min.

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

Immundiagnostik AG recommends to use a pre-column to extend the lifetime of the column.

9. TREATMENT OF THE COLUMN

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

10. RESULTS

Calculation

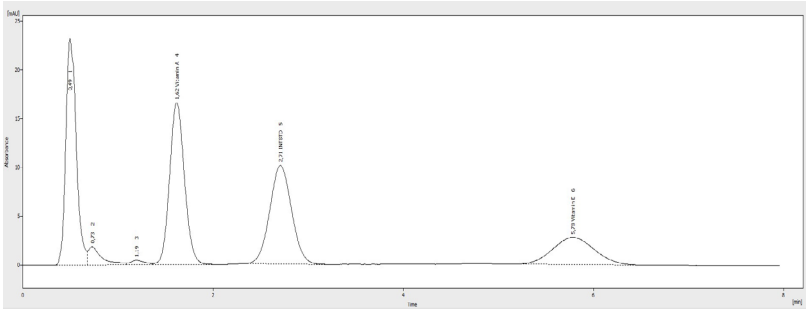
$$\frac{\text{Peak height sample} \times \text{concentration standard*}}{\text{Peak height internal standard in the sample}} \times F = \text{concentration of the sample}$$

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

* see label

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

Typical chromatogram



11. LIMITATIONS

Strongly haemolytic as well as lipaemic samples sometimes show wrong concentrations. We therefore advise against measuring such samples.

12. QUALITY CONTROL

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 analysed 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 acceptance 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**) and internal standard (**INTSTD**) must be disposed of 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 too old	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 conveys inaccurately	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 conveys inaccurately	Check pump, degas the system

Problem	Possible reasons	Solution
Baseline is not calm	Pump conveys inaccurately	Check pump, degas the system
	Detector flow cell is dirty	Clean flow cell

16. PRECAUTIONS

- Calibrator (**CAL**) and controls (**CTRL1** and **CTRL2**) are based on human plasma. They have been tested for HIV and hepatitis B and found to be negative. However, as a precaution, the test components should always be handled as potentially infectious material.
- The test components contain organic solvents. Avoid contact with skin or mucous membranes.

17. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- All reagents in the kit package are for research use only.
- Do not use reagents beyond the expiration date stated on the kit label.
- Do not interchange different lot numbers of any kit component within the same assay.
- Do not mix plugs and caps from different reagents.
- Follow the guidelines for laboratories.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which has not been consulted with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be lodged within 14 days after receipt of the product. The product should be sent to Immundiagnostik AG along with a written complaint.
- Analyse controls with each run.
- Always perform the assay according to the enclosed manual.
- 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

19. SYMBOLS


Temperature limitation

Catalogue number

For research use only

To be used with


Manufacturer

Content 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

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